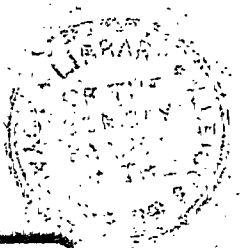
Report of the Haffkine Institute

For the Year 1937

133222

BY

LT.-COL. S. S. SOKHEY, I.M.S.
Director, Haffkine Institute



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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1937.

PART I.

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Department of Pharmacology.

Dr. B. B. Dikshit, Ph.D. (Edin.), M.R.C.P. (Edin.), D.P.H. (Cal.).

Dr. B. K. Nandi, Ph.D., M.Sc.

Department of Bio-Chemistry.

Mr. M. A. Malandkar, M.A., M.Sc.

Mr. S. K. Gokhale, B.A., M.Sc. A.I.I.Sc.

Anti-Rabic Department.

Lieut. Hugh Maurice, I.S.O., I.M.D.

Mr. R. K. Gokhale, L.C.P.S., S.M.S.

Anti-Plague Inquiry.

Mr. R. G. Sathe, G.B.V.C., Veterinary Assistant Surgeon.

Voluntary Research Workers.

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Mr. R. Sadashivan, B.Sc.	... Do.
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Mr. R. B. M. Colah, B.Sc.	... (Diagnostic Department).
Mr. A. J. Naidu ...	... (Pathology).
Mr. R. D. Panwalkar, A.M.	... (Artist and Photographer).
Mr. M. V. Kamat, B.A.	... (Librarian).

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Mr. D. S. Dixit ...	... Store-keeper.
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Mr. S. Y. Nabar ...	... Packing.

Junior Staff, Subordinates and Inferior Establishment number 102.

GENERAL REVIEW.

During the year under review (1937), as in previous years, the limited resources of the Institute were almost entirely devoted to the routine production of large quantities of prophylactic vaccines. The Institute produces Haffkine Plague Vaccine in large quantities to meet the needs of the whole of India, and prepares Cholera, Typhoid and Antirabic vaccines for use in the Province of Bombay. In addition, the Institute functions as the Provincial Bacteriological Laboratory for the Province, and does a considerable amount of diagnostic work for the Government hospitals. Though the Institute has no resources of its own for conducting researches, important investigations in plague and pharmacological studies of synthetic anti-malarials were conducted with the generous aid of the Indian Research Fund Association. A number of voluntary workers also carried out a number of researches.

Department of Vaccines and Sera.

During the year there was increase in the incidence of plague in the country, 33,460 attacks of plague were recorded as compared with 23,873 attacks during the previous year. This increased incidence called for correspondingly large quantities of plague vaccine. Altogether 13,24,337 doses (i.e. 3,973,011 c.c.) of plague vaccine were issued during the year. Haffkine Plague Vaccine is still playing a decisive role in the fight against plague in India, and the demand for the vaccine continues to be high in relation to the incidence of the disease.

The active campaign initiated a few years ago against the spread of cholera at public fairs again made large demands for cholera vaccine, and 6,40,978 doses of the vaccine were issued during the year. In addition, 10,779 doses of meningococcal vaccine and 9,325 doses of typhoid vaccine were issued. Meningococcal vaccine has been issued during the last few years purely as an experimental measure. It was hoped that it would be possible to collect data which would throw some light on the value of this vaccine. Unfortunately no worthwhile data have materialised.

The supply of anti-toxins and therapeutic sera to Government hospitals has been kept up and is proving a success.

Plague Researches.

(1) *Plague Vaccine.*—A considerable amount of work was reported in the last report on the effect, on the resultant vaccine, of (*a*) the temperature of incubation of cultures (27°C and 37°C), (*b*) the medium in which cultures are grown (broth and agar), and (*c*) the method of killing cultures. Heat-killed vaccine was also compared with live avirulent vaccine. The plan was to make four measurements of the protective power of each vaccine under study. But at the time of reporting four measurements for all the vaccines had not been completed. During the year the work was continued and the necessary determinations completed. Another difficulty had also been experienced, that is, the proper evaluation of the experimental results obtained. Reed and Muench's method of estimating

50 per cent. end-points was found suitable for the work, and consequently the minimum mouse protective dose has been calculated by this method. These extended observations confirm the previous tentative conclusions. In the case of broth vaccines, those incubated at 27°C. are more potent than those incubated at 37°C. While in the case of agar vaccines, those incubated at 37°C. are more potent than those incubated at 27°C. Killing of cultures by the application of heat at 55°C. for 15 minutes gives better vaccines than when the cultures are killed with phenol or formalin.

One new observation of some interest has emerged. As reported before we had developed a method of rendering plague strains avirulent by repeatedly subculturing them on agar slopes at weekly intervals and incubating them at 37°C. By this method we had rendered avirulent strain 120/5H. The live avirulent vaccine used in our previous study was made from this strain and it proved to be possessed of very high protective value. During the year we rendered avirulent another strain, strain I, by the same method. But the live avirulent vaccines prepared from this strain have proved to be almost entirely devoid of any protective power. This would seem to show that avirulent strains differ in their antigenic value. We propose to study this point more fully. The behaviour of avirulent strains in this respect should be compared with the behaviour of virulent strains. We have not noticed any measurable differences in the antigenic value of the large number of different virulent strains we have used in our work. If a suitable vaccine is made from a fully virulent strain it always seems to possess a very high protective power.

(2) *Anti-Plague Serum.*—Production of the serum on the lines reported before was continued, but no opportunity arose during 1937 of testing the serum in the field. However, early in 1938, a small epidemic occurred at Bettiah, Bihar, and with the generous assistance of Bihar Government and the co-operation of the Director of Public Health, Bihar, we were enabled to deal with 124 cases. We shall publish full details in the next report, but it may be mentioned here that cases treated with serum gave a percentage mortality of 27 and the controls a percentage mortality of 65; the patients were taken alternately for serum treatment and as controls without selection.

(3) *Epidemiology of Plague.*—An interesting fact has emerged from our studies which seems to have a bearing on the mechanism of pandemics of plague, how they come to an end and how they start again after the lapse of time. The Indian Plague Commission working in 1907, when plague was still raging in Bombay, found that the rodents responsible for the spread of plague in the City were *Rattus rattus* and *Rattus norvegicus*, which formed over 99 per cent. of the rat population. They found these species to be highly susceptible. They also found that *Gunomys varius* was not a common rodent in Bombay City and that it formed only 1 per cent. of the rat population. We, working in 1937, find that *Rattus rattus* and *Rattus norvegicus* together form only about 70 per cent. of the rat population and *Gunomys varius* accounts for

the remainder 30 per cent. More interesting still, *Gunomys varius* is highly susceptible to plague infection while the other two species are now highly resistant, that is, the erstwhile agents of the spread of the disease have become resistant and are being replaced by a species which is highly susceptible.

Department of Pharmacology.

The department continued its investigations into the pharmacology of synthetic anti-malarials and its studies in acetylcholine. The previous observation that Plasmoquine did not affect the course of pregnancy in any way was extended by the observation that placenta acted as a filter and prevented the drug from getting into fœtal circulation. By an ingenious technique it was shown that while toxic doses of the drug definitely affected the maternal heart, it showed no effect in the heart tracing of the fœtal heart. The measurement of the concentration of the drug in the maternal blood stream and in the fœtal circulation confirmed the above observation. Similar studies on the action of Atebrin are also in progress.

Studies in acetylcholine have yielded further interesting facts. The role played by this substance n the transmission of nerve impulses at nerve ends in muscles is well known, but it was doubtful whether it played a similar role in the central nervous system at the ends of the afferent nerves. Dr. Dikshit has been able to obtain some experimental evidence that acetylcholine is liberated in the central nervous system when afferent nerves are stimulated.

With a further generous grant from the Indian Research Fund Association the department was strengthened by the employment of a highly trained research chemist, Dr. B. K. Nandi, who has facilitated the pharmacological studies of plasmoquine by developing a new test for quantitative and qualitative estimation of the drug in tissues. Two voluntary research workers Dr. H. S. Mahal and Miss K. Bhagvat made studies in choline esterase and developed an enzyme method for the estimation of adrenaline.

This department is still entirely maintained by the Indian Research Fund Association and is engaged primarily in researches on synthetic anti-malarial drugs, but it is hoped Government will take over the department and strengthen it to enable the department to take up work in connection with the control of the sale of drugs in the Province.

Biochemical Department.

The department besides its routine work continued work on establishing normal clinical standards for Indians. For establishing red cell and hæmoglobin standards for normal Indian men and women 121 men and 101 women between the ages of 18 and 30 years had been examined. Hæmoglobin determinations were made by Van Slyke oxygen capacity method, a method which yields very accurate results. An average of 15·37 g. of hæmoglobin per 100 c.c. of blood for men and an average of 12·99 g. for women

were obtained. Contrary to all expectation these averages tallied exactly with similar hæmoglobin averages worked out in America for American subjects in spite of the dissimilar dietetic and climatic conditions of Indian and American subjects. This observation points to the possibility of there being a physiological constant for hæmoglobin. The subject is being studied.

Diagnostice Department and the Department of Public Health Chemistry and Bacteriology.

The departments dealt with over 9,000 specimens sent by hospitals and dispensaries and carried out over 5,000 Wassermann and Kahn tests. Proposals have been submitted to Government to throw open the services of these departments to a larger public.

Anti-Rabic Department.

The work of this department is steadily increasing. During the year 14 new treatment centres were opened in the Province, bringing up the total number of centres to 83. This increase in the number of centres called for a larger quantity of vaccine and 9,715 treatments (i.e. about 680,050 c.c. of the vaccine) were issued during the year.

This year again showed a large increase in the number of patients who received treatment at the Institute itself, 3,498 patients. Evidently stray dogs are also on an increase in the city. This menace to the health of the public should be abated by a more systematic and sustained efforts at the destruction of stray dogs in the City.

Income and Expenditure.

This year again the Institute managed to pay its way and earned a small surplus of Rs. 36,681 for the calendar year. Table on page 34 gives detailed figures.

SOCIAL LIFE AT THE INSTITUTE.

The Critics Club plays an important part in the collective scientific and social life of the Institute. During the year a number of social functions were arranged and at the various scientific meetings held, the following papers were read :—

(1) The Value of certain Calcium Caynide Preparations for Fumigating Rat Burrows and Dwellings, by Dr. Chitre.

(2) Plasmoquine in Pregnancy, by Dr. Dikshit.

(3) Precipitin Reaction as a Measure of the Potency of Anti-Plague Serum, by Dr. Wagle.

(4) A new Chemical Method for detection and estimation of Plasmoquine in Tissues, by Dr. Nandi.

(5) Recent Work on Pernicious Anæmia Factors, by Dr. Wills.

The Critics Tennis Club and the Haffkine Institute Sports Club continue to grow in popularity. The membership of both clubs increased during the year. Matches for the Mackie Cup and the Sokhey Cup were well patronised and the tournaments were a great success. To the games of Tennis, Cricket, Hockey, Football (Socker) and Badmington the game of Volleyball was added.

The lunch room which was started last year has proved a success. It is well patronised and meets a long felt need of the staff for their midday meal and afternoon tea away from their work rooms.

Haffkine Institute Co-operative Credit Society Limited—This is the second year of the society and it has already proved of great benefit to the members of the staff. The Society encourages thrift and issues loans to members at a very low rate of interest on a co-operative basis. The books and accounts of the society were examined by the Registrar of Co-operative Societies during the year and the society declared a first class society.

SECTION I.

Vaccine and Serum Department.

A. *Haffkine's Plague Vaccine.*

1,324,337 doses of plague vaccine were issued during the year against 881,053 doses in the previous year.

The number of doses of plague vaccine despatched monthly during the years 1936 and 1937 are detailed below:—

	1936.	*1937.*
January	92,957	132,937
February	77,522	141,074
March	104,134	116,278
April	15,748	91,915
May	775	14,009
June	48,383	18,601
July	53,897	24,423
August	65,668	219,169
September	92,984	206,912
October	70,926	48,629
November	82,474	203,860
December	175,585	106,530
	881,053	1,324,337

From the opening of the Laboratory to the end of 1937 a total of 42,234,708 doses has been issued. The output of the vaccine in quinquennial periods has been as follows:—

Years.	Doses.
1897-1901	2,867,041
1902-1906	3,455,537
1907-1911	3,584,262
1912-1916	4,567,366
1917-1921	6,667,894
1922-1926	7,202,636
1927-1931	5,929,243
1932-1936	6,636,392
1937	1,324,337
	42,234,708

The geographical distribution of the vaccine in India and to places outside India during the years 1936 and 1937 is given below:—

	Number of doses.	
	1936.	1937.
British India.		
Bombay Presidency	59,833	106,867
Madras Presidency	116,537	2[illegible]3,4[illegible]3
Bengal, Bihar and Assam	20,128	40,[illegible]24
United Provinces of Agra and Oudh	156,4[illegible]8	278,183
Punjab	33,540	5,628
North-West Frontier Provinces	79	135
Central Provinces and Berar	22,624	52,060
Delhi Province	1[illegible]3	282
Sind	2,840	17,339
Port Blair	32	.....
	412,271	714,851

	Number of doses.	
	1936.	1937.
Indian States.		
Southern Maratha States	6,483	21,566
Hyderabad, Deccan	154,000	256,695
Mysore	97,648	212,972
Patiala	784	156
Kashmere	5,040	.....
Jaipur	5	103
Jodhpur	36	48
Cambay	96	.....
Bhopal	1,216	392
Indore	864	8
Cochin	1,968	1,200
Rampur	256	.....
Rewa	40	.....
Bharatpur	192	.....
Baroda	.....	1,488
Gwalior	.....	4,032
Sawantwadi	.....	304
Rajkot	.....	12
Junagad	.....	400
Benaras State	.....	8,155
	268,628	507,532
Foreign Countries.		
Burma	193,120	90,816
China	592	.....
Ceylon	152	400
Aden	5,040	5,040
Federated Malay States	250	1,062
Cairo, Egypt	1,000	1,400
Iraq	.....	3,236
	200,154	101,954
Total number of doses ...	881,056	1,324,337

B. *Cholera Vaccine.*

Cholera vaccine is prepared largely for the use of the Health Department of the Province. With greater efforts which are being made to keep the outbreaks of the disease under check large quantities of the vaccine continue to be used. During the year 640,978 doses of the vaccine were issued. This is the fourth year in succession which shows a large consumption of the vaccine. The provision of facilities to the people of the Province for getting themselves inoculated at their home town dispensary before proceeding to large fairs where outbreaks of the disease usually occur should prove an important factor in suppressing the outbreaks of the disease.

The large productions of the vaccine are throwing a great strain on the limited staff of the Institute, which will have to be augmented before long to enable the work to be carried on.

Cholera vaccine issued month by month from 1935 to 1937.

	1935.	1936.	1937.
January ...	2,070	11,191	7,958
February ...	4,439	12,801	5,650
March ...	4,945	4,553	11,688
April ...	3,305	4,341	33,655
May ...	7,227	21,364	87,339
June ...	8,110	192,563	41,235
July ...	91,970	118,183	207,945
August ...	127,112	92,487	135,100
September ...	94,259	64,173	49,360
October ...	24,634	55,370	19,453
November ...	19,430	33,330	11,168
December ...	5,530	95,265	30,427
	393,031	705,621	640,978

C. *Other Vaccines.*

Statement of vaccines issued monthly during the year 1937.

	Mixed T. A. B. Vaccine in ccs.	Meningococcal Vaccine in ccs.
January ...	805	115
February ...	69	4,500
March ...	211	375
April ...	143	1,525
May ...	740	445
June ...	1,365	.....
July ...	746	4
August ...	367	.....
September ...	1,873	375
October ...	1,670	.....
November ...	691	45
December ...	645	3,395
	9,325	10,779

D. *Sera.*

During the year under report the following sera were issued by the Institute :—

		Ampoules.
1.	Anti-Tetanic Serum (Prophylactic) 1,500 International Units, 1½ cc. ...	5,740
2.	Anti-Tetanic Serum (Curative) 20,000 International Units, 10 cc. ...	1,805
3.	Anti-Diphtheria Serum (Prophylactic) 1,000 International Units, 1 cc. ...	304
4.	Anti-Diphtheria Serum (Curative) 10,000 International Units, 10 cc. ...	198
5.	Anti-Dysenteric Serum 8,000 International Units, 20 cc.	864
6.	Anti-Streptococcus Serum Polyvalent, 10 cc.	1,206
7.	Anti-Menigococcus Serum, 10 cc. ...	336
8.	Anti-Gas gangrene Serum, 10 cc. ...	620
9.	Normal Horse Serum, 5 cc.	38
10.	Normal Horse Serum, 10 cc. ...	502

SECTION II.

Epidemiology of Plague.

This department carried out a number of routine duties besides researches into Plague Epidemiology narrated in Part II of the report.

A.—*Training of Health Personnel.*

1. At the request of the Director of Public Health for the Government of Bombay, nine Epidemic Medical Officers, namely, Messrs. V. K. Vatve, R. M. Naik, B. V. Kulkarni, D. V. Keskar, P. V. Kulkarni, K. K. Karnik, G. S. Pendse, K. V. Borkar and A. S. Saiyad, were given a course of training in rat dissection for detection of plague lesions.

2. Captain P. N. Sathe, M.B.B.S., B.Hy., D.P.H., A.I.R.O., a special officer of Bombay Health Department was given a course of training in Plague Preventive measures including fumigation with calcium cyanide compounds.

3. The 1937 class of Sanitary Inspectors, divided into four batches, was given a short course of demonstrations and lectures in the subject of rat examination and rat and flea destruction.

4. Eight members of the Post-graduate class in Tuberculosis were given demonstrations of the methods of rat examination and rat destruction.

5. Seven women students attending the Bombay Health Visitors Class were given demonstrations.

6. At the request of Sind Government, Rao Bahadur G. D. Chitre, L.M. & S., B.M.S. (I), visited Karachi City for about a week. During this period he gave instructions to the local Sanitary Officers on the detection of plague infection in rats. He also explained to them the method to be adopted for collecting samples of local rats for determining their relative anti-plague immunity and samples of rat fleas for ascertaining the general and specific flea indices of the local rat population. The Sind Government also detailed Dr. Sayed Ahmaduddin, M.B.B.S., to the Institute for a month for training in plague preventive measures.

B.—*Examination of Bombay City Rats.*

The examination of rats and other rodents of Bombay City sent by the Executive Health Officer of the Bombay Municipality for evidence of plague was continued during the year. The Municipality maintains a permanent establishment for catching rats and about 2,000 rats are caught every night in the City and they are marked with the locality from where they are caught. Out of these rats, those which arrive at the Institute dead are post-mortemed and examined for plague. In doubtful cases smears and cultures are made. This study, besides keeping the Municipality informed of any plague epizootic in any particular part of the City, is providing valuable information on the subject of plague epidemiology. Tables summarizing the monthly figures of rats received at this Institute and the number out of them that were examined are put up herewith. It will be seen that no plague-infected rat was found during the year.

Since the month of June all the rodents received alive were also classified by species. The result of these observations is given in Tables Nos. I, II, III and IV.

TABLE I.

Rats received at the Institute from the Municipality during 1937.

Months.	Alive.	Dead.	Total.
January	39,225	26,662	65,887
February	33,793	24,036	57,829
March	34,582	23,329	57,911
April	29,876	26,948	56,824
May	28,593	25,159	53,752
June	28,448	22,572	51,020
July	29,869	26,439	56,308
August	23,277	27,906	51,183
September	26,153	27,179	53,332
October	41,107	32,038	73,145
November	28,437	33,979	62,416
December	30,180	34,063	64,243
Total ...	373,540	330,310	703,850

TABLE II.

Examination of Dead Rats for Plague during 1937.

Months.	Dead rats examined.	Found plague infected.	
		Rattus norvegicus.	Rattus rattus.
January	14,554	Nil.	Nil.
February	11,839		
March	11,553		
April	16,636		
May	12,897		
June	12,669		
July	16,245		
August	16,651		
September	15,405		
October	19,896		
November	19,270		
December	19,372		
	186,987		

TABLE III.

Relation of Human Plague Deaths to Plague Deaths among Rats during 1937.

Months.	Human plague deaths.	Rats examined.	Plague infected rats.
January	0	14,554	0
February	0	11,839	0
March	0	11,553	0
April	0	16,636	0
May	0	12,897	0
June	0	12,669	0
July	0	16,245	0
August	0	16,651	0
September	0	15,405	0
October	0	19,896	0
November	0	19,270	0
December	0	19,372	0
	0	186,987	0

TABLE IV.

TABLE

Table giving the Classification of Rats

Mon

Species.	June 1937.		July 1937.		August 1937.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
Rattus rattus	4,282	18·0	4,867	18·3	4,766	25·2
Rattus norvegicus	6,207	26·1	7,058	26·6	4,037	21·5
Gunomys varius	8,706	36·6	8,729	32·9	6,059	32·1
Bandicota indica	254	1·1	246	0·9	143	0·8
Mus musculis	1,239	5·2	1,162	4·4	1,189	6·3
Crodidura coerulea	1,302	5·5	2,102	7·9	1,108	5·9
*Unidentifiable	1,754	7·5	2,396	9·0	1,551	8·2
†Total ...	23,747	100·0	26,560	100·0	18,676	100·0

*Rats included under this head, though reported to have been caught alive, were received

†These totals do not agree with the totals of live rats in Table I because rats received on

IV.

trapped alive in the City of Bombay.

thly.

September 1937.		October 1937.		November 1937.		December 1937.		For whole period.	
Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
5,058	24·7	12,781	39·5	5,785	27·0	4,761	22·3	42,310	25·7
5,062	24·7	7,309	22·6	5,253	24·5	5,502	25·8	40,448	24·5
6,661	32·5	8,319	25·7	5,697	26·6	6,517	30·6	50,688	30·7
130	0·6	191	0·6	183	0·9	219	1·0	1,366	0·8
928	4·6	939	2·9	1,230	5·7	1,048	4·9	7,728	4·7
1,220	6·0	1,226	3·8	1,163	5·4	935	4·4	9,056	5·5
1,418	6·9	1,609	4·9	2,125	9·9	2,344	11·0	13,191	8·1
20,477	100·0	32,365	100·0	21,436	100·0	21,326	100·0	164,787	100·0

at the Institute dead, often mutilated and even putrid.
Sundays and holidays were not classified.

C. *Examination of Rats from Steamers in the Bombay Harbour.*

The Health Officer of the Port of Bombay forwarded 193 rats (89 killed and 104 alive) from 18 steamers. Out of the former 37 (41·6 per cent.) were *Rattus rattus,* and 52 (58·4 per cent.) were *Mus musculis.* Amongst the 104 live rodents, 72 (69·2 per cent.) were *Rattus rattus,* 8 (7·7 per cent.) were *Rattus norvegicus,* 1 (1·0 per cent.) was *Gunomys varius,* 22 (21·1 per cent.) were *Mus musculis* and 1 (1·0 per cent.) was unidentifiable. Flea count was done on 98 out of the 104 living rodents. These yielded altogether 4 fleas namely an average of 0·04 flea per rat. Two out of these four fleas were *X cheopis* (one male and one female) and the remaining two *X astia* (both females). All the above rats except two were examined for plague but none was found plague infected.

D. *Examination of Rats from other sources.*

During the year 103 rats were received for examination from different towns in the Province. Of these the carcases of four were too putrid for examination. The remaining 99 were examined and two of them were found plague infected. In addition smears of 17 other rats were also received. Of these, one showed presence of plague bacilli.

E. *Preparation and Supply of Exhibits on Plague and other Diseases.*

The Department prepared several exhibits during the year and puppled them to various bodies for use in Medical Schools or for ublici exhibitions. Specimens showing lesions of plague were supplied to the Professor of Bacteriology, Grant Medical College and Professor of Pathology and Bacteriology, Seth Goverdhandas Sunderdas Medical College, Parel, Bombay. Also mounted specimens of fleas were supplied to the Medical Officer of Epidemics, Cochin State, and to the Principal, Medical College, Vizagapatam.

F. *The Manufacture of approved Rat Traps.*

The following manufacturers were supplied during the year with scale designs of the approved rat trap best suited to conditions in India and were given general assistance in the matter:—

(*a*) Yeravda Central Prison, Yeravda, Poona District.

(*b*) Imperial Sanitary Goods Work, Garha via Jallundur Cantonment.

G. Exhibits such as Placards, Photos, Posters on Plague, Malaria, Guinea-worm, etc., were supplied to the following :—

(1) The International Rural Hygiene Conference held at Bandœng (Java).
(2) The Medical Officer of Health, Poona City Municipality.
(3) Nutan Marathi Vidyalaya High School, Poona.
(4) Health Section of Poona, Industrial Exhibition, 1937.
(5) Baby Week Show, Bassein.
(6) Bandra Baby and Health Week.

SECTION III.

Department of Pharmacology.

The activities of the department during the year under review were the same as detailed in the annual report last year. Due to a generous grant from the Indian Research Fund Association, it was possible to engage a research Chemist and some laboratory attendants in the department. Dr. B. K. Nandi, Ph.D., has been working in the department as the research chemist since the end of April 1937 and has been engaged in the investigation of some chemical problems in relation to the pharmacological action of antimalarial remedies.

Two voluntary research workers, Dr. H. S. Mahal, Ph.D., and. Miss K. Bhagvat, M. Sc., worked in the department for some months Dr. Mahal completed his investigation on the Pharmacological action of certain Flavones and the results were published in the proceedings of the Indian Academy of Sciences. He showed that these Flavones do not possess any antiseptic or anthelminthic action. Dr. Mahal has also been associated with the work on Choline esterase, as well as some investigations on the distribution of Atebrin in the tissues. The details of these investigations are given elsewhere.

Miss Bhagvat joined the department in March 1937 and worked till August 1937 when she proceeded to England for further studies. She worked out a new enzyme method for assaying adrenaline and having compared this method with the standard biological method, showed that the new method compared very favourably with the biological one. She also worked on the digestibility of proteins of certain Indian food stuffs and has already collected some important data on the subject.

The work on anti-malarial drugs such as Plasmoquine and Atebrin was carried out, as already stated, under the auspices of the Indian Research Fund Association. Investigations on the action of Plasmoquine in pregnancy were continued. It was demonstrated last year that the drug could be given in pregnancy without much danger of inducing an abortion. The effects of the drug on the fœtus are now under investigation and it appears that the placenta filters out Plasmoquine and prevents it from reaching the fœtus.

Experiments on the concentration of Atebrin in the circulating blood were made and it was found that the dye diffused out very rapidly into the tissues. It was further observed that the concentration of the dye in blood remained approximately the same although the dose and method of administration of the dose varied.

A new colorimetric test for detection and estimation of Plasmoquine in the tissues was developed in the laboratory. The new test can detect Plasmoquine up to a dilution of 1 in a million and can be used for quantitative estimation of the drug up to a limit of 1 in 200,000.

Investigations on the action of acetylcholine were continued. The study of formation of acetylcholine by tissues has now been undertaken and it has been shown that the Pancreas can form considerable quantities of acetylcholine. It was further observed that the guineapig's intestines formed greater quantities of acetylcholine than any other tissue of this animal except the brain.

Experiments on the role of choline esterase in varying physiological conditions were completed by Dr. Mahal and the paper has been accepted for publication in the Indian Journal of Medical Research.

The fact that toxæmias reduce the Choline esterase concentration of the blood was demonstrated in guineapigs by inducing plague infection in these animals. The results were published in the Quarterly Journal of Exprimental Physiology, London. Attempts to prove chemical transmission in the Central nervous system were continued and some indirect evidence has been obtained which suggests that such transmission is possible. These observations are discussed in Part II.

Biological Assay of Drugs.—This laboratory now accepts drugs like Digitalis, Strophanthus, Squill, Ergot and Pøst. Pituitary Extract for biological assay. One sample of Tincture Digitalis manufactured locally was tested and was found to be weak in strength, being considerably below the international standard.

Miscellaneous work :—One drug which is very popular as an anti-malarial remedy in the province was tested for its anti-malarial action in monkeys and was found to have no anti-malarial property at all. Several drugs reputed to be snake bite cures were also tried and were proved to possess no curative or protective power against Cobra or Viper bites.

SECTION IV.

Department of Bio-chemistry.

This Department makes available to hospitals and medical practitioners the chemical analytical methods in the diagnosis of disease. The routine diagnostic work carried out is detailed in the table below. In addition research work was continued for establishing normal standards for Indians for the various substances for which blood and urine are subjected to analysis for diagnostic purposes. A new study on ("a possible hæmoglobin constant" was started. Details of researches are given in Part II of this report.

Routine Examinations carried out in 1937.

Blood—		*Cerebro-spinal Fluid—*	
Nonprotein Nitrogen	93	Total Protein	23
Urea Nitrogen	140	Globulin	12
Uric acid	29	Sugar	21
Creatinine	22	Chlorides	18
Cholesterol	34	Urea	3
Chlorides	16	Nonprotein Nitrogen	2
Ammonia Nitrogen	10	Lange's Colloidal Gold Test	20
Sugar	52		99
Phosphorus	6		
Serum calcium	15		
Hæmoglobin	33		
Cell Volume	21		
Indices	20	*Fæces—*	
Van den Bergh Reaction	10	Fat in fæces	5
	501	Occult blood	7
			12
Urine—			
General Qualitative test	56		
Total Nitrogen	15		
Urea Nitrogen	52	*Miscellaneous—*	
Uric acid	11	Glucose Tolerance Test	15
Sugar	50	Kidney Function Test	43
Chlorides	3	Blood Sedimentation Test	6
Ammonia Nitrogen	6	Coagulation Time of Blood	2
Albumin	11	Gastric analysis	11
H ion concentration	4	Basal metabolism	33
Total acidity	3	Fluids, pleuritic, cystic etc.	3
Calcium	2		113
Lactose	1		
Special protein	1		
Blood	2		
Lead	7		
Urinary Calculus	1		
	225	Total	950

SECTION V.

Department of Public Health Chemistry and Bacteriology.

A. Chemical Work.

Waters.—During the year under report 15 samples of water were examined, majority of these being for sanitary purposes. The chemical composition of these waters showed extreme variations in total solids (192-24) and chlorides (64·0-2·0) as compared to the recognised chemical standards of purity.

B. Bacteriological Work.

Waters.—During the year under review the following samples were examined :—

Drinking waters	11
Waters from Swimming Baths	6
Total ...	17

For drinking waters the method outlined in Report No. 71 of the Ministry of Health on the Bacteriological Examination of waters was followed.

C. Miscellaneous Work.

Five consignments of plants imported from abroad were fumigated with hydrocyanic acid gas. This work was undertaken on behalf of Customs Department.

Stains and standard solutions were prepared and supplied to various laboratories of this Presidency.

SECTION VI.

Diagnostic Department.

A. Bacteriological Section.

The routine work carried out is summarised in the following table.

Material examined.	Positive.	Negative.	Total.
A Blood—			
(*a*) Films for microscopical examination—for malaria	2	33	35
„ filaria ...	...	1	1
„ differential leucocytic count ...	...	...	48
(*b*) Count for R.B.Cs. ...	...	...	30
„ „ W.B.Cs. ...	...	...	22
„ „ Platelets ...	...	...	2
(*c*) Cultures for organism (B. Typhosus, etc.)	5	15	20

Material examined.	Positive.	Negative.	Total.
(*d*) Serological examinations—			
Wassermann Reaction ...	951	1,945	3,138 (228 indefinite, 14 anti-complementary.)
Kahn Reaction ...	771	1,868	2,812 (173 indefinite.)
Blood grouping ...	...	...	9
Agglutination tests—			
for enteric group ...	114	152	266
,, undulant fever ...	1	6	7
,, Weil-Felix reaction ...	1	1	2
,, B. colic ...	...	1	1
,, B. Dysentery ...	9	2	11
,, B. Dysentery with ascetic fluid	3	...	3
B. Sputum—			
Microscopical examination for tuberculosis.	6	19	25
Cultural examination for various micro-organisms.	8	...	8
C. Urine—			
General chemical and microscopical examinations.	...	...	264
Microscopic examination for Gonococci ...	...	2	2
Cultural examinations ...	135	41	176
Animal test for tuberculosis ...	3	8	11
Friedman test for pregnancy ...	3	2	5
D. Fæces—			
(*a*) Microscopical examination for ova, cysts and amœbæ.	55	87	142
(*b*) Cultural examination—			
for cholera, typhoid and dysentery ...	10	37	47
,, miscellaneous organisms ...	...	...	14
(*c*) Microscopical examination and animal experiments for T. B.	...	3	3
(*d*) Examination for occult blood ...	...	2	2
E. Cerebro-spinal Fluid—			
(*a*) Microscopical examination—			
for T. B. and other organisms ...	1	19	20
,, cell count ...	...	...	27
(*b*) Cultural examination ...	2	16	18
(*c*) Wassermann reaction ...	10	43	55 (2 indefinite.)

Material examined.	Positive.	Negative.	Total.
F. Pus—			
Microscopical and cultural examinations...	15	5	20
G. Miscellaneous microscopical examination of smears—			
Smears for B. diphtheria ...	1	6	7
„ „ Gonococcus ...	...	6	6
„ „ Leprosy ...	11	11	22
„ „ Spirillum of rat-bite fever.	...	1	1
„ „ Tubercle bacillus and other pathogenic organisms from pleuritic and other cyst fluids.	2	5	7
„ „ Leishman donovan bodies.	...	1	1
H. Miscellaneous cultural examination—			
Swabs for B. diphtheria ...	1	6	7
„ „ various organisms ...	...	...	21
Fluids for pathogenic organisms ...	2	5	7
Foodstuffs for organisms of Salmonella group.	...	3	3
Cuttings from clothing for cholera (medico-legal cases).	...	8	8
I. Miscellaneous animal tests—			
B. diphtheria ...	1	...	1
Spirillum of rat-bite fever ...	...	1	1
J. Miscellaneous work—			
Disinfectants for carbolic coefficients .	...	...	2
Identification of snakes ...	...	...	8
„ „ worms ...	...	...	1
K. Supplies—			
(1) *Vaccines—*			
(*a*) Autogenous vaccines ...	...	...	65
(*b*) Bacterial emulsions ...	...	...	5694 cc.
(2) *Sera—*			
Blood grouping sera ...	...	...	24 „
Kahn's antigen ...	...	...	25 „
(3) Living cultures of pathogenic micro-organisms.	...	...	65
(4) *Media—*			
Loeffler's Serum Agar tubes ...	...	...	258
Blood agar tubes ...	...	...	650

B. Pathological Section.

During the year under review 758 pathological tissues were received for examination. The following table gives a rough classification of the nature of these tissues and their number :—

——	No.	Per cent.
Non-malignant tumours	54	7·1
Malignant tumours	87	11·5
Tissues found to be normal	2	0·3
Miscellaneous pathological conditions (including research specimens).	367	48·4
Received in a state unfit for examination	4	0·5
Animal brains for Negri bodies	244	32·2
Total ...	758	.....

Of the 87 malignant tumours, 55 were carcinomas, 12 epitheliomas, 2 endotheliomas, 2 malignant melanomas, 9 sarcomas, 3 seminomas, 2 teratomas and 2 rodent ulcers. One of the melanomas was of rectal origin, a rather rare site for a melanoma. Of the two teratomas, one had its origin in ovary, and the other in testicle. Rodent ulcers were confined to face. Thirty out of 55 carcinomas occurred in female reproductive organs, breast being the commonest site. Besides this the female generative system contributed to one case of chorionic epithelioma—sarcomas arose from common sites.

It would not be out of place to mention here a difficulty that this department often experiences in the routine examination of pathological specimens. Hospitals or medical men submitting specimens for examination do not in many instances supply adequate clinical history of the case with the result that in many cases it becomes extremely difficult to form a correct opinion. Writing and re-writing for more information necessarily involves loss of time and delay in the submission of reports. Attention to this point would greatly facilitate the work of this department.

SNAKES AND SNAKE VENOM.

Snakes.

The following snakes were received during the year 1937 :—

Cobras	10
Russell's Vipers	52
Kraits	48
Echis carinata	539

Venom Supplies.

Cobra	23·25	grammes.
Russell's Viper	22·25	„

Solutions of Venoms supplied.

Cobra venom solution varying from 5 to 25 mouse units supplied in 1 cc. ampoules ...	857	ampoules.
Russell's Viper venom solution, 1 in 10,000, in 1 cc. ampoules	55	„

SECTION VII.

Anti-Rabic Department.

Nine thousand seven hundred and fifteen treatments (i.e. about 680,050 ccs. of 5 per cent. vaccine) were issued during the year, against 7,570 treatments issued during the previous year (1936). This large increase is due to 14 new treatment centres having been opened in the Province during the year. The total number of out-centres now is 83. The new anti-rabic centres were opened at the following stations :—

Bombay Province.

(1) Civil Hospital, Satara.
(2) Civil Hospital, Bijapur.
(3) Civil Hospital, Dhulia.
(4) Civil Hospital, Alibag.
(5) Civil Hospital, Kaira.
(6) Civil Hospital, Broach.
(7) Civil Hospital, Dharwar.
(8) Civil Hospital, Godhra.
(9) Civil Hospital, Thana.

Indian States.

(1) State Hospital, Brijnagar, Jhalwar State, Rajputana.

Other Provinces.

(1) Civil Hospital, Khandwa.
(2) Civil Hospital, Chindwara.
(3) Civil Hospital, Chanda.

Railway.

(1) Railway Hospital, Dohad (B. B. & C. I. Railway).

The treatments carried out at the Institute itself form a bettet controlled group both as regards the technique of treatment and Health Returns of the treated cases after the treatment. These figures are given in the table below.

The details of the treatments carried out at the Institute in 1936 and 1937 are summarised as below :—

	1936.	1937.
Number of persons applied for treatment .	2,961	3,498
Advice Cases	622	473
Those who completed full treatment ...	2,331	3,014
Percentage of Health Returns ...	98·7%	Awaited.
Cases died of Hydrophobia	2	Do.
Total Mortality Rate	0·08%	Do.

The figures show a large increase of persons bitten by stray dogs. That it should be so is a serious reflection on the city. A large number of stray dogs continue to roam in the streets. More vigorous and systematic destruction of these dogs should be undertaken to abate this menace.

Table showing the number of brains received and examined at the Institute for evidence of Rabies during the year 1937.

—	Number.	Percentage.
Received in a state unfit for examination	4	1·6
No evidence of Rabies	46	18·9
Positive for Rabies, " Negri Bodies present "	194	79·5
	244	.....

Statement showing the classification of brains and different places from where the brains were received.

—	Positive.	Negative.	Unsuitable for examination.	Total.
Received through the Bombay Veterinary College.	76	8	0	84
Received through the Bombay Police.	21	0	0	21
Received from private owners in the City.	6	0	0	6
Received from private owners in the Province.	5	3	0	8
Received through Out-Centres .	86	35	4	125
Total ...	194	46	4	244

Statement showing the classification of different animals with the result of their brain examinations for Rabies.

——	Positive.	Negative.	Unsuitable for examination.	Total.
Dogs	189	44	4	237
Goat	1	0	0	1
Jackals	2	0	0	2
Buffalo	1	0	0	1
Cow	1	0	0	1
Horse	0	1	0	1
Donkey	0	1	0	1
Total ...	194	46	4	244

SECTION VIII.

Income and Expenditure.

The following table shows the income and expenditure of the Haffkine Institute for the year 1937 :—

Receipts.	Rs.	Expenditure.	Rs.
XXVIII-Public Health—		39-D-Public Health—	
Sale proceeds of Sera and Vaccines etc.	3,62,575	D-Bacteriological Laboratories—	
Miscellaneous	2,125	Haffkine Institute—	
		Pay of Officers ...	76,671
		Pay of Establishment ...	77,197
		Allowances, Honoraria, etc.	11,178
		Contingencies ...	1,49,773
		Total ...	3,14,819
		39-E-Public Health—	
		E-Pasteur Institutes—	
		Pay of Officers	11,600
		Allowances, Honoraria, etc.	1,600
		Total ...	13,200
Total Receipts ...	3,64,700	Total Expenditure ...	3,28,019

PART II.

RESEARCH WORK.

I. Plague Vaccine.

(SOKHEY.)

In the last report a considerable amount of work was reported on the role played in the preparation of plague vaccine by (*a*) the temperature of incubation of culture (27°C. and 37°C.), (*b*) the medium in which cultures are grown (broth and agar), (*c*) the virulence of the organism and (*d*) the method of killing cultures. Vaccines prepared from heat-killed virulent cultures and live avirulent were also compared. At the outset it had been decided to make four determinations of the protective power of each vaccine under study by the standardised methods described in the 1932-35 Report. But at the time of reporting in a number of instances four determinations had not been completed. During the year work was continued to complete four determinations for each vaccine. Considerable difficulty in correctly evaluating the results of these measurements had been experienced in previous years. The statistical method described in the Medical Research Council Report No. 183, 1937, and recommended to us was found to be too time consuming for our purpose. But while writing up the results of this year's work a simpler statistical method for estimating fifty per cent. end points by Reed and Muench came to our notice (Reed, L. J. and Muench, H., American Journal of Hygiene, 1938, 27, 493). We have found this method to be eminently suited for our work, and have used it for calculating results reported below. In the case of each vaccine 100 mice were used for the four measurements carried out and five graduated doses of each vaccine were used, in the case of one vaccine, however, 250 mice were used.

A. *The relative Protective Power of Broth Vaccines incubated at 27°C. and those incubated at 37°C.*

Broth Vaccines.	Minimum Mouse Protective Dose.
Vaccine 120*/5H (15th September 1936) incubated at 27°C. for four weeks (four Experiments)	0·0025 c.c.
Vaccine 120/5H (15th September 1936) incubated at 37°C. for four weeks (four Experiments)	0·0080 c.c.
Vaccine I (21st October 1936) incubated at 27°C. for four weeks (four Experiments) ...	0·0021 c.c.
Vaccine I (21st October 1936) incubated at 37°C. for four weeks (four Experiments) ...	0·0043 c.c.

In all cases growths were killed at 55°C. for 15 minutes, and carbolised to contain 0·5 per cent. phenol.

*This strain through typographical error, was designated as 125H in the last report. The correct number is 120/5H.

B. *The Relative Protective Power of Agar Vaccines incubated at 27°C. and those incubated at 37°C.*

For these experiments a 48-hour broth culture of a given strain was planted on nutrient agar slopes. These were incubated for 48-hours, some at 27°C. and others at 37°C. Suspensions were then made with saline and standardised to contain 1,500 million organisms per c.c. Growths were killed at 55° C. for 15 minutes and then phenol added (0·5 per cent.). The number of 1,500 million organisms was fixed by actual experiment, so that the best agar vaccine may have about the same protective power as the best broth vaccine.

Agar Vaccines.	Minimum Mouse Protective Dose.
Vaccine 120/5H (24th August 1936) incubated at 27°C. (4 Experiments)	0·0851 c.c.
Vaccine 120/5H (24th August 1936) incubated at 37°C. (10 Experiments)	0·0017 c.c.
Vaccine I (26th August 1936) incubated at 27°C. (4 Experiments)	0·0590 c.c.
Vaccine I (26th August 1936) incubated at 37°C. (4 Experiments)	0·0033 c.c.

C. *Live Avirulent Broth Vaccines compared with Heat-killed Broth Vaccines.*

In the previous report we had described a method of maintaining the virulence of plague cultures for long periods, and also a method for making cultures avirulent. We were thus enabled to have the same strain both in a fully virulent and in an avirulent form. Using the virulent and the avirulent forms of strain 120/5H we have made broth vaccines, incubated for 4 weeks. Temperatures of incubation employed were 27°C. and 37°C. The vaccines made from virulent strains were first killed at 55°C. for 15 minutes and then phenol added (0·5 per cent.).

Broth Vaccines.	Minimum Mouse Protective Dose.
Heat-killed Vaccine 120/5H (15th September 1936) incubated for 4 weeks at 27°C. (4 Experiments)	0·0025 c.c.
Live Avirulent Vaccine 120/5H (26th September 1936 and subsequent dates) incubated for 4 weeks at 27°C. (4 Experiments) ...	0·0096 c.c.
Heat-killed Vaccine 120/5H (15th September 1936) incubated for 4 weeks at 37°C. (4 Experiments)	0·0080 c.c.
Live Avirulent Vaccine 120/5H (26th September 1936) incubated for 4 weeks at 37°C (4 Experiments)	0·0038 c.c.

D. *Live Avirulent Agar Vaccines compared with Heat-killed Agar Vaccines.*

The strains used in the experiments detailed above, under C, were used in this experiment. The cultures were grown for 48 hours on nutrient agar slopes and suspensions made in saline. These were standardised by colony counts to contain 1,500 million organisms per c.c. The virulent cultures were killed at 55°C. for 15 minutes and phenol added (0·5 per cent.).

Agar Vaccines.	Minimum Mouse Protective Dose.
Heat-killed Vaccine 120/5H (24th August 1936) grown at 27°C. (4 Experiments) ...	0·00851 c.c.
Live Avirulent Vaccine 120/5H (14th April 1937 and subsequent dates) grown at 27°C. (4 Experiments)	0·1175 c.c.
Heat-killed Vaccine 120/5H (24th August 1936) grown at 37°C. (10 Experiments) ...	0·0017 c.c.
Live Avirulent Vaccine 120/5H (12th December 1936) grown at 37°C. (4 Experiments) ...	0·0008 c.c.

E. *Effect of the method of killing cultures on the potency of the resultant vaccine.*

Broth Vaccines.	Minimum Mouse Protective Dose.
Incubated at 27°C. for 4 weeks—	
Vaccine 120/5H (15th September 1936) killed at 55°C. for 15 minutes and then added phenol, 0·5 per cent. (4 Experiments) ...	0·0025 c.c.
Vaccine 120/5H (15th September 1936) killed at 60°C. for 1 hour and then added phenol, 0·5 per cent. (4 Experiments)	0·0097 c.c.
Vaccine 120/5H (15th September 1936) killed by the addition of phenol, 0·5 per cent., no heat applied (4 Experiments)	0·0028 c.c.
Vaccine 125/5H (15th September 1936) killed by the addition of formalin 0·025 per cent., no heat applied (4 Experiments) ...	0·0046 c.c.
Incubated at 37°C. for 4 weeks—	
Vaccine 120/5H (15th September 1936) killed at 55°C. for 15 minutes and then added phenol, 0·5 per cent. (4 Experiments) ...	0·0080 c.c.
Vaccine 120/5H (15th September 1936) killed at 60°C. for 1 hour and then added phenol, 0·5 per cent. (4 Experiments)	0·0159 c.c.
Vaccine 120/5H (15th September 1936), killed by the addition of formalin, 0·025 per cent. (4 Experiments)	0·0093 c.c

Agar Vaccines.	Minimum Mouse Protective Dose.
Incubated at 27°C. for 48 hours—	
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c.. killed at 55°C. for 15 minutes and then added phenol, 0·5 per cent. (4 Experiments) ...	0·0851 c.c.
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c., killed at 60°C. for 1 hour and then added phenol 0·5 per cent. (4 Experiments) ...	0·3000 c.c.
Vaccine 125/5H (24th August 1936), suspension contained 1,500 million organisms per c.c., killed by the addition of phenol, 0·5 per cent. no heat applied (4 Experiments) ...	0·0650 c.c.
Incubated at 37°C. for 48 hours—	
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c., killed at 55°C. for 15 minutes and then added phenol, 0·5 per cent. (10 Experiments) ...	0·0017 c.c.
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c., killed at 60°C. for 1 hour and then added phenol, 0·5 per cent. (4 Experiments) ...	0·0016 c.c.
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c., killed by the addition of formalin, 0·025 per cent., no heat applied (4 Experiments) ...	0·0019 c.c.
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c., killed by the addition of phenol, 0·5 per cent. (4 Experiments)	0·0020 c.c.

F. *Vaccines made from different avirulent strains compared.*

Last year we had worked with strain 120/5H made avirulent by repeated (64) weekly subcultures at 37°C. During the year we had made strain I avirulent. This strain had needed 80 weekly subcultures to make it avirulent. 75th subcultures still showed a little but measurable virulence. From this avirulent strain we have made several broth and agar vaccines and have tried to determine the minimum mouse protective dose. In the case of three agar vaccines incubated 37°C. we have given as much as 0·05 c.c. per mouse and even this dose failed to protect. In the case of broth vaccine incubated 27°C. as much as 0·8 c.c. failed to show any protection. While in the case of 120/5H strain as little as 0·0008 c.c. of 37°C. agar vaccine and 0·0096 c.c. in 27°C. broth vaccine was found to be the minimum mouse protective dose.

This observation is interesting. Both the strains 120/5H and I were fully virulent to start with and they were both made avirulent by the same method. Yet the behaviour of the two resulting avirulent strain is totally different; strain 120/5H has retained its protective properties, while strain I seems to have lost its antigenic value altogether. We propose to study this problem further. We have made two more strains avirulent for this study.

II. Anti-plague Serum.

(SOKHEY.)

1. *Preparation of the Serum.*—Work on the lines reported in the last report was continued. All along a serious difficulty has presented itself in the effective immunisation of horses. The only method that we have at present of measuring the potency of the serum at any stage of immunisation is the white mouse protection test described in the 1932-1935 report of this Institute. But this method takes a month to obtain a reading. During this period while the protective power of a serum is under measurement only two courses are open to us, either to stop the further immunisation of a horse or to continue giving it further injections. In either case the potency of the serum changes either for better or worse and we have no means of knowing in which direction the change is proceeding, and this change is often so great that very often when we have bled a horse on the basis of the determination started a month previously we have obtained very poor sera, sometimes of course also very much better sera. But in any case this is a serious handicap. During the year with the help of the Indian Research Fund Association the staff of the enquiry was strengthened with a view to work out quantitative precipitin and aglutinin reactions to see if these quickly applied reactions can solve the practical difficulty. This work is still at a preliminary stage.

2. *Comparative protective value of different batches of sera produced.*—During the year seven different batches of sera were produced and their protective power was measured and the following results obtained :—

	Minimum Mouse Protective Dose.
No. 12 (1)	0·043 c.c.
No. 12 (3)	0·047 c.c.
No. 12 (4)	0·058 c.c.
No. 13 (1)	0·050 c.c.
No. 13 (2)	0·028 c.c.
No. 16 (1)	0·050 c.c.
No. 16 (2)	0·050 c.c.

The average protective dose works out in the neighbourhood of 0·050 c.c.; only in one case did we get a more potent serum (0·028 c.c.).

3. *Field Tests.*—No opportunity occurred during 1937 for trying out our serum in the field, but early in 1938 a mild epidemic occured at Bettiah, Bihar. The Bihar Government built a temporay plague hospital to enable the serum to be tested under controlled conditions. A total of 124 cases of plague were dealt with. The patients were taken alternately on admission (*a*) for serum and (*b*) for control treatments. During the third week of the experiment no controls were kept because of an outcry against the selection of patients for serum treatment. However, this difficulty was overcome and the experiment continued, but as a result the number of serum treated patients were 69 and controls 55. The control treatment consisted of intravenous injections of iodine solution. A full note on the experiment will be published in the next report, but it may be noted for the present that out of 69 serum treated cases 19 died, giving a percentage mortality of 27 and out of the 55 controls 36 died giving a percentage mortality of 65.

III. Serology of Plague.

A. Precipitin Reaction.

(WAGLE.)

Work on the precipitin reaction with a view to see the possibility of its serving as an index to the potency of anti-plague sera was continued. Last year the tests were done with two kinds of antigen, one extracted at 37°C. from a saline suspension of plague-bacilli and the other at 100°C. When the sera were tested with the latter antigen, the titres obtained showed a close correlation with the results of the Biological test. Thus the sera of Horses Nos. 12 and 13 which had a titre of one in 3,200, also gave good protection to experimentally infected white mice, while other sera e.g., those of Horses Nos. 7, 14, 15, 16, 17, 18 and 19 which had a titre of less than 1 in 200 did not show any such protective action.

As immunization proceeded further however, and samples were tested from time to time by both the methods, it became apparent that a close correlationship seen between the results of the two tests in the beginning was not being maintained. For, while the two horse sera 12 and 13 continued to give high titre of precipitation along with good protection in experimentally infected mice, results obtained by the two tests with some of the other Horse sera would not show similar correlation. Thus horse 6 serum which at one stage of immunization showed a precipitin titre of 1 in 3,200, did not give equally good protection in mice, while Horse 16 serum, which showed a titre of 1 in 800 was found to have a good protective act on. These results made it clear that we were not getting a very correct idea as to the potency of the sera from the test employed.

It was thought that titration of serum by the optimal proportions method would give a more accurate idea about the precipitin content of the sera. This test depends on the principle that if the antigen and antibody are mixed in certain definite proportions, flocculation

will take place earliest in the tube in which the reactants are in optimum proportions. When either the antigen or antibody is present in relative excess the reaction is delayed. Employing this method three horse sera which were found to be potent biologically, e.g., Horse sera 12, 13 and 16 were titrated. It was found that while Horse sera 12 and 13 showed two zones of flocculation by this method, Horse 16 serum always gave one flocculating zone. The following table will show the presence of two zones, wherein the titration of Horse No. 13 is shown. In this experiment, the quantity of serum added to each tube was the same, but the quantity of antigen added varied :—

Readings taken after.	Each tube contains 1 cc. of (1 in 10) Horse 13 serum and 1 cc. of the respective dilution of antigen.									Antigen control (1 cc. of undiluted antigen + 1 cc. Saline).	Antiserum control (1 cc. of 1·10) Horse 13 serum + 1 cc. of Saline.
	Dilutions of antigen extracted at 100°C.										
	Undiluted.	1·2	1·5	1·10	1·20	1·50	1·100	1·200	1·400		
Immediately after mixing.	c	c	c	c	c	c	c	...	...	...	...
20 Min. ...	c	c	c	c	c	c	c	$c^?$	...	...	...
30 Min. ...	f	c	c	c	c	P^1	P	$c^?$	...	...	...
35 Min. ...	F	c	c	c	?	f^2	f^1	$c^?$	...	...	...
40 Min. ...	FF	p	?	?	p	f^4	f^2	p	...	...	...
Ratio of antigen to serum ...	10:1	5:1	2:1	1:1	1:2	1:5	1:10	1:20	1:40		

c means cloudiness present in the tube but no particles.

p means very small particles visible in the tubes with the aid of handlens.

P means bigger particles visible in the tubes with the aid of handlens.

f means small floccules visible in the tubes with the aid of handlens.

F means small floccules visible in the tubes with naked eye.

FF means large floccules visible in the tubes with naked eye.

Numericals added to letters show the relative size of particles or floccules, for instance f^2 is bigger than f^1 and so on.

Sign...means absence of both cloudiness precipitate in the tubes.

The explanation of these two zones was not quite clear; Goldsworthy and Taylor have observed the occurrence of these zones and Taylor considered that these separate zones represented separate reactions of different components of proteins against their specific antibodies. Question naturally arose whether the sera showing two zones had more than one antibody present in them. The first probability that suggested itself was the presence of anti-body against agar in addition to anti-bodies against *B. pestis*. The occurrence of anti-bodies against agar in the horses' sera has been

reported upon by other workers, and during the immunization of horses against plague, there was ample opportunity for agar to go into the horses' system by way of saline emulsions of *B. pestis* prepared for intravenous injections. The sera were therefore tested for the presence of agar anti-bodies by putting them with a saline solution of agar and it was found that both the sera (Horse 12 and 13) which showed double zones showed also the presence of agar antibodies. Horse No. 16 Serum which showed only a single zone of precipitation did not show presence of any agar antibody.

Before, therefore, dealing further with the method of optimal proportions and the double zones of precipitation, it was decided to see whether a titre of plague antibodies obtained by removal of agar antibodies from the serum would afford a better idea as to the potency of the serum. It was clear that the precipitin titre obtained originally with the different sera was a combined titre of plague and agar antibodies, and therefore to obtain a titre of plague antibodies only, it was necessary to absorb from the serum agar antibodies, and then to test it for its plague-antibody content. When tested in this way, the various sera gave the following results :—

Horse Sera.	Titre obtained.	Biological Test.
6	1 in 400	Not potent.
12	Between 1 in 600 and 1 in 800 ...	Potent.
13	1 in 800	Do.
14	Between 1 in 400 and 1 in 600 ...	Not potent.
15	1 in 400	Do.
16	1 in 800	Potent.
17	Between 1 in 400 and 1 in 600 ...	Not potent.
18	Do. do. ...	Do.
19	1 in 600	Do.

It will be seen, that sera which are potent biologically, show a precipitin titre higher than that of other sera which are found to be weak. But the differentiation between the weak and strong sera does not seem to be very great.

This method will be further explored, and the optimal proportion method fully studied in the current year.

B. The application of Shwartzman reaction for titration of antiplague serum.

(WATS.)

The work to develop some satisfactory serological method for titration of the antiplague sera, prepared at this Institute, has been carried on. The discovery by Shwartzman (J. Exp. Med., 48, 247,

1928) of the phenomena of local cutaneous reaction to bacterial filtrates has offered a new biological reaction by means of which a soluble antigenic toxin can be precisely measured and the anti-toxic serum titrated by its ability to neutralise or prevent specifically the biologic effect of the toxin.

Technique.—The original technique developed by Shwartzman has been followed with slight modifications. After 48 hours cultures at 27° and 37°C on the appropriate medium the growth was emulsified in normal saline (25 c.c. per Roux bottle). The emulsion was filtered first through Keisulghur filter paper and then through L3 candle. The filtrate (0·25 c.c.) was injected intradermally into the abdominal skin of a rabit. The hair from the skin, 24 hours previous to the injection, were removed by means of a depilatory powder. An intravenous injection of the same filtrate was given 24 hours after the intradermal injection the dose being 1 c.c. per kilogramme of the body weight. The animal was kept under observation for 24 hours.

The filtrates for testing were obtained by growing 5 virulent strains of *B. pestis* on " acid agar ", blood agar, cystine agar gelatin digest agar, and nutrient agar, for 48 hours at 27°C and 37°C respectively. Filtrates were also obtained by growing the above strains of *B. pestis* in acid digest broth for 2, 14, 28 and 56 days at 27°C respectively. These filtrates were used as such and also concentrated in vacuo at a temperature of 42° to 44°C before use. The results of all the above were negative, there being no skin reaction.

Jonkow—Weregnekow and Lepatova (Trop. Dis. Bull. 31, 12, p. 886, 1934) were successful in obtaining Shwartzman's phenomena in rabbits " by means of the envelop culture of *B. pestis* emulsified in distilled water and lysed by subsequent freezing and thawing ". This experiment was repeated using CO_2 snow for freezing and a water bath at 42°C for thawing. This process was repeated 20 times for each experiment. The results are negative in all cases except for a slight erythema after the intradermal injection.

Precipitation test.—The precipitation test has been used as an index of potency of several antisera. To obtain the flocculation or precipitation in very high dilutions the isolation of the specific non-protein fraction (bacterial polysaccharides) seems to be necessary. Dingle (Amer. J. Hyg. 20, 1, July 1934, p. 148) isolated a carbohydrate fraction from Pasteurella cuniculicida and as *B. pestis* belongs to the same group this technique was adopted with a few modifications. The ultimate product gave Molish reaction and negative Biuret test as in the case of Dingle, but did not show any precipitation with the antiplague sera of known therapeutic potency. This experiment was repeated with similar results.

An attempt was also made to prepare a fraction by submitting the acetone killed bacilli to trysin degestion at 37°C at ph. 8·5 according to the method of Raistrick and Topley (Brit. J. Exp. Path. 15, 113, 1934). The digest was centrifuged, filtered through

L_3 candle and the fraction precipitated by addition of 68 per cent. alcohol. The final product did not give any precipitation or other immunological reactions with the anti-plague serum.

Casper (J. Immunology 32, 6, 1937, page 421) working with gonococci found trypsin digestion unsatisfactory but used 20 per cent. sodium taurocholate as more satisfactory. This method was also tried with similar negative results.

IV. Epidemiology of Plague.

(SOKHEY AND CHITRE.)

1. *Susceptibility to Plague of Rats from different parts of India.*—In the previous two reports considerable amount of work was reported on the relative susceptibility to plague infection of rats from different places in India which had suffered from plague epidemics of varying intensity sometime or other during the present pandemic. It was shown that the percentage of deaths from plague among rats after experimental infection had a rough inverse ratio to the aggregate incidence of plague at the place from which rats were collected. It was hoped to complete this survey by covering the Province of Bengal, the only province not yet covered. Arrangements were made to obtain rats from Calcutta City, Dacca City, Berhampur, Burdwan, Rangpur and Patna. After a great deal of correspondence and some expenditure we managed to procure 24 rats from Berhampur only, no rats from other selected places could be obtained. It became clear that it would not be possible to get rats from these places unless we sent our own staff down to collect rats. This was found to be too expensive and the enquiry has for the time being abandoned and data are being collected for publication.

The examination of rats from Berhampur gave the following results :—

Name of Province.	Name of City or Town.	Aggregate human plague death rate per mille (1899-1938).	Percentage death rate from experimental standardised plague infection.
Bengal ...	Berhampur ...	0·00	62·5
Bombay ...	Bombay (as control of immune rats).	199·88	20·0
Madras ...	Madras (as control of susceptible rats).	0·396	90·0

2. *Natural Resistance of rats to plague infection.*—In the previous report experiments were reported which seemed to show that there existed in nature strains of rats which were resistent to plague infection. A number of batches of rats obtained from Dharmsala, a hill station in the Punjab, where no plague epidemic

has been known to have occurred during the present pandemic were examined and it was found that an appreciable percentage of these resisted experimental plague infection. To further verify this question we obtained two batches of rats (*Rattus rattus*) from Simla, Punjab, and Berhampur, Bengal, two stations where little or no plague epidemic had been known to have occurred. Over 30 per cent. of the rats from these stations resisted experimental infection. These results correspond with the observations previously made on rats from Dharmsala and lend further support to the hypothesis that the resistence shown by rats in places where plague epidemics, and therefore plague epizootics, have occurred is due to the fact that plague epizootics destroy strains of rats susceptible to plague infection leaving behind naturally resistent strains.

The following results were obtained :—

TABLE No. I.

Susceptibility to Experimental Plague Infection of Rats from Simla and Berhampur.

Name of Town.	Aggregate human plague death rate per mille (1899-1929).	Number of rats from locality used for.	Death from experimental plague infection.	Percentage deaths.
Simla	0·28	15	10	66·7
Controls—				
Bombay	199·88	20	2	10·0
Madras	0·396	20	16	80·0
Berhampur	0·00	24	15	62·5
Controls—				
Bombay	199·88	10	2	20·0
Madras	0·396	10	9	90·0

3. *Susceptibility to Plague of different species of Rats.*—In the last report another interesting observation was just mentioned. It was found that while the Bombay house rat (*Rattus rattus*) was highly resistent to experimental infection, another rat, *Gunomys varius*, also found in houses in Bombay was highly susceptible. Since then we have made further observations which confirm the fact. We also have determined the susceptibility to plague infection of Bombay *Rattus norvegicus* and find this rat is as highly resistent as the *Rattus rattus*.

TABLE No. II.

Table showing percentage deaths from standardised experimental plague infection among rats of different species caught in the City of Bombay.

Name of City or Town.	Percentage deaths from experimental plague infection.		
	Rattus rattus.	*Rattus norvegicus.*	*Gunomys varius.*
Bombay	5·0	7·5	69·2
Madras (as controls)	90·0	.....	.....
Bombay	0·0	.....	75·7
Madras (as controls)	75·0	.....	.....
Bombay	10·0	5·0	.....
Madras (as controls)	90·0	.....	.....

In connection with these observations another interesting fact emerges. During 1907 when the plague epidemic was still active in Bombay, the Indian Plague Commission noted that *Nasokia bengalensis* (*Gunomys varius*) was not a common rodent in Bombay City and that it formed only 1 per cent. of the rat population of the City and that the rest of the rat population was made up almost entirely of *Mus rattus* (*Rattus rattus*) and *Mus decumanus* (*Rattus norvegicus*) ; *Mus rattus* forming 66·2 per cent. of the rat population. During 1937, June to December, we have classified 164, 787 rats collected in the City of Bombay (see Table IV pages 16 and 17) and find that *Gunomys varius* now (1937) forms over 30 per cent. of the rat population while *Rattus rattus* forms only about 25, 7 per cent. Some of the difference between the observations carried out in 1907 and 1937 may well be due to personal factor in classification and possibly methods of trapping. The Indian Plague Commission also found, working in 1907, that both *Rattus rattus* and *Rattus norvegicus* were equally and highly susceptible to experimental infection. They found that 45 per cent. of the rats tested succumbed to infection. While, in 1937, find that both *Rattus rattus* and *Rattus norvegicus* are highly resistent to plague, (10 per cent. susceptible), and have in course of time been replaced to a considerable extent by *Gunomys varius* which is highly susceptible (about 70 per cent. susceptible). This is an interesting observation and may have some bearing on the mechanism of pandemics, how they come to an end and how they start again after a lapse of time.

V. Estimation of Adrenaline by Enzyme Method.

(BHAGVAT.)

Miss Bhagvat observed that the oxidase prepared from the seeds of *Dolichos lablab* Linn has the property of oxidising adrenaline to a red coloured compound. The intensity of the colour was proportional to the quantity of adrenaline present and hence the method was employed to estimate adrenaline in fresh suprarenal glands. The results obtained by this method were compared with those obtained by the biological method and were found to be in very good agreement. A paper describing the details has been accepted for publication in the Indian Journal of Medical Research.

VI. Pharmacology of Plasmoquine.

(DIKSHIT.)

A preliminary note on the action of Plasmoquine in pregnancy was published in the Records of Malaria Survey of India and described the action of toxic doses of this drug on the course of pregnancy. These observations were extended and the effects of therapeutic doses of the drug in various stages of pregnancy studied. It was found that therapeutic doses did not interrupt the normal course of pregnancy in any way. The litter given by mothers who had one or more courses of plasmoquine during pregnancy did not in any way differ from the normal litter. The contractions of the foetal heart were recorded graphically while the foetus was still retaining its maternal connections and it was observed that a dose of plasmoquine which considerably affected the mother's heart, when injected into the mother, did not in any way disturb the action of the heart of the foetus. These experiments strongly suggest that the placenta acts like a filter and prevents large quantities of the drug reaching the foetus. These results were read before the symposium on malaria held at Calcutta.

VII. Studies in Acetylcholine.

(DIKSHIT.)

Attempts to demonstrate liberation of acetylcholine in the central nervous system as a result of stimulation of the central ends of the vagus nerves were partially successful. The problem was tackled in dogs by severing the nervous connections of the head with the body, while the vascular connections were kept intact as far as possible. To achieve this end the spinal cord was sectioned at the level of third cervical vertebra in the neck and the two vagus nerves cut. Stimulation of the central ends of the cut vagus nerves did not produce any effect on the blood pressure under these conditions, but if the animal was given large doses of eserine (with a protective dose of atropine) such stimulation produced a rise in blood pressure. This was assumed to be due to liberation of acetylcholine in the brain, which under the protection afforded by eserine could circulate and produce its pressor effect in the

atropinized animal. The rise in blood pressure produced by a minute's stimulation was of the order of a rise that could be produced by about 25γ of Acetylcholine under similar conditions.

VIII. Formation of Acetylcholine by tissues.

(DIKSHIT.)

Tissues of different animals were allowed to respire in oxygenated Locke's solution containing 1 per cent. glucose end kept at body temperature. The choline esterase activity was inhibited by adding eserine to make a concentration of about 1 in 5,000. It was found that the brain, intestines and urinary bladder of dogs formed considerable quantities of acetylcholine. The acetylcholine formed was assayed on the Frog's *Rectus abdominis* muscle. Bubbling nitrogen instead of oxygen considerably lessened the formation of acetylcholine by the brain, but affected only slightly that formed by the intestines.

IX. Choline esterase in toxæmias.

(DIKSHIT AND MAHAL.)

Previous observations by other workers that the choline esterase concentration in the blood of a given individual remains constant for long periods were repeated and confirmed. Guinea-pigs, for example, were found to possess the same quantity of choline esterase in a given volume of blood for months. Some guinea-pigs were then infected with plague and the amount of choline esterase at the height of infection determined. It was found that such infection markedly lowered the choline-esterase concentration in guinea-pig's blood. These experiments were confirmed by one experiment in man. The enzyme concentration of the blood was determined on 3 different days and found to be the same. A large dose of Haffkine's Plague Vaccine was then given which produced a considerable reaction. An examination of the blood at the height of reaction showed a considerable reduction in the quantity of the enzyme. The enzyme concentration returned to normal after complete clinical recovery. These results were published in the Quarterly Journal of Experimental Physiology, London.

X. Pharmacology of Flavones.

(MAHAL.)

The antiseptic and anthelminthic properties of 6 different flavones synthesized by Dr. Mahal in the Royal Institute of Science were studied. None of these showed any such action which could be of any practical use. The general pharmacological actions of these flavones were also studied and it was found that they inhibited the movements of the isolated rabbits gut and uterus in concentration of about 1 in 140,000. They also lowered the blood pressure, increased respiration and contracted the uterus in intact anæsthetized animals, after intravenous injections of doses varying

from 20 to 40 mg. p. Kg. The isolated frog's heart was inhibited by concentrations varying from 1-100,000 to 1-500,000. These results were published in the Proceedings of the Indian Academy of Sciences.

XI. Studies on blood Choline esterase.

(MAHAL.)

The Choline esterase activity of the blood of different species of animals was determined by titration, gasometric and pharmacological methods. Frog's *Rectus abdominis* muscle was used for the latter. It was found that the activity varied in different species and different individuals of the same species, but was constant for the same individual. The amount of the enzyme present was the same in the arterial and the venous blood. The blood retained its choline esterase activity for 48 hours if stored in the refrigerator but lost it considerably if heated at a temperature above 45°C. Starvation or feeding glucose did not affect the enzyme activity nor did such physiological variation as sleeplessness or changes in the surrounding temperature. The action of some drugs was also studied and it was found that hypodermic administration of large doses of drugs like strychnine, morphine, acetylcholine and cobra venom did not modify the choline esterase activity of the blood. This work has been accepted for publication in the Indian Journal of Medical Research.

XII. Choline ester formation by Pancreas.

(MAHAL AND DIKSHIT.)

Quastel has demonstrated the formation of a Choline ester by the Cortex of rat's and guinea-pig's brain. His method was followed to study the formation of choline ester by the dog's brain and pancreas. It was found that the ester formation was greater in the case of the brain than the pancreas, but the pancreas could form considerable quantities of the ester even in the absence of oxygen, while the brain could not. It was further observed that in the absence of oxygen the pancreas formed H_2S. A simultaneous determination of the choline esterase activity of these organs showed that the pancreas contained a large amount of the enzyme. These observations were published in a small note in the Current Science.

XIII. A colour test for Chromates and Dichromates.

(NANDI.)

While investigating a colour test for Plasmoquine, it was observed that chromates and dichromates gave a rich purple colour when brought in contact with Plasmoquine in the presence of oxalic acid at a pH below 6·5. Chromates and dichromates could be detected by this test up to a dilution of 1 in 100,000. The test is specific and with a given concentration of Plasmoquine the colour intensity is proportional to the amount of chromates and dichromates present. A note on this test was published in the Current Science.

XIV. A colorimetric test for Plasmoquine.

(NANDI AND DIKSHIT.)

This new test is an application of Folin's Phenol reagent to Plasmoquine. When a few drops of the reagent are added to an aqueous acid solution of Plasmoquine and the mixture made strongly alkaline by the addition of sodium carbonate, a blue colour develops, which deepens into its maximum intensity in about half hour. This test can detect Plasmoquine up to a dilution of 1 in a million and can be used for quantitative estimation up to 1 in 200,000. The test is not specific for Plasmoquine and a number of substances in the tissues which possess a phenolic character respond to this test. The test however can be extended to detect and estimate plasmoquine in tissues by treating the latter with an excess of alkali (NaOH) and extracting with ether. Under these conditions all phenolic compounds in the tissues are converted into their sodium salts, which are highly soluble in water and insoluble in ether. Control experiments with normal tissues of various animals uniformly gave negative results when tested in this manner with the phenol reagent. In tissues containing plasmoquine, however, a positive result was obtained and if the concentration of the drug was sufficiently high, it could be estimated colorimetrically. This test could not be extended for detection of plasmoquine in the urine.

XV. A Hæmoglobin Constant.

(SOKHEY AND MALANDKAR.)

For establishing red cell and hæmoglobin standards for normal Indian men and Indian women, 121 men between the ages of 18 and 30 years and 101 women of the same age group were examined at the Haffkine Institute. All the subjects examined belonged to the Province of Bombay. Men subjects were medical students and women subjects were either medical students or nurses. All the hæmoglobin determinations were made by the Van Slyke Oxygen Capacity method.

An average of 15·37 g. of hæmoglobin per 100 c.c. of blood for men and an average of 12·99 g. for women were obtained. It was found that these averages tallied exactly with hæmoglobin averages for white men and women of the same age group, worked out in the United States of America by the same method. American averages for men were—Haden 15·34 g., and Dill 15·38 g.; American average for a group of women was 12·93 g. It is obvious that the American subjects lived under totally different climatic conditions to those which prevail in India, and also the dietetic habits of the American subjects were very dissimilar to those of the Indian subjects. In spite of these dissimilar dietetic and climatic conditions the hæmoglobin averages for the American and Indian subjects were the same. This observation raised the question of a possible hæmoglobin constant.

An investigation has been started to explore this possibility. Subjects of the same age group as were examined in the previous determination are selected for the present investigation. Total

quantity of hæmoglobin in the body is determined to see if it bears any definite ratio to the various body units employed in the determination of Basal Metabolic Rate, such as surface area according to Du Bois, body weight actual and that calculated for sitting height and chest circumference according to Dreyer's directions and Stoner's formula and standing height. Total quantity of hæmoglobin in the body is determined from the determination of hæmoglobin—grammes per 100 c. c. of blood by Van Slyke Oxygen Capacity method and the total volume of circulating blood by the dye method of Keith, Rowntree and Geraghty.

Work done so far gives encouraging results and it shows that there is a close relationship between the surface area of the body and the total quantity of hæmoglobin. Five cases out of the 8 examined so far have a relationship of 349 g. to 358 g. per square metre of body surface, while for 6 cases the range was 322 g. to 358 g. per square metre of body surface. Two cases gave figures higher than these. These early results are encouraging enough to make it worth our while to continue the work.

XVI. Nonprotein nitrogenous constituents of blood in the laked and unlaked blood filtrates.

(GOKHALE.)

The bloods of eighteen healthy young men were examined. The total nonprotein nitrogen and urea nitrogen, uric acid creatinine, total creatinine and amino acid nitrogen were estimated in both the laked blood filtrates prepared according to the Folin-Wu technique and the new unlaked blood filtrate prepared according to the new technique by Folin (Jour. Biol. Chem. *86*, 173, 1930; *88*, 85, 1930). The averages of the results obtained are given in the following table:—

Constituent.	In laked Blood Filtrate.		In unlaked Blood Filtrate.	
	mg. per 100 c. c.	mg. N. per 100 c. c.	mg. per 100 c. c.	mg. N. per 100 c. c.
Total Nonprotein Nitrogen ...	30·38	.....	18·23	.....
Urea Nitrogen	12·98	12·98	12·63	12·63
Uric Acid	2·88	0·96	2·74	0·91
Creatinine	1·36	.....	1·13	.....
Total Creatinine	3·38	1·26	1·43	0·53
Amino acid Nitrogen ...	6·38	6·88	3·93	3·93
Total N. P. N. by addition ...	.....	22·08	.....	18·00
Total N. P. N. found ...	.....	30·38	.....	18·23
Difference ...	.....	8·30	.....	0·23

The above results show that when analysis is done on unlaked blood filtrate, the sum of the nitrogen of the constituents of the nonprotein nitrogen tallies with the total nonprotein nitrogen within experimental error. This is not the case with the analysis of laked blood filtrate.

XVII. Coagulation time of blood and concentration of Russell's Viper Venom.

(GOKHALE.)

A solution (1 in 100) of dry venom was made in 0·85 per cent. saline and successive dilutions prepared from it. To 0·1 c.c. of the venom solution kept in a paraffined glass tube (75 mm. × 10 mm.) 1 c. c. of fowl plasma (obtained according to the technique of Dr. Trevan of the Welcome Physiological Laboratories, London) was added and the time required for coagulation of the plasma observed. Experiments were done at 37°C. The following table gives the average of the results of the experiments:—

Dilution.	Amount of venom in 0·1 c. c. mg.	Time for coagulation of 1 c. c. of Fowl Plasma.	
		Minutes.	Seconds.
1 in 1,000	10^{-1}	0	26
1 in 10,000	10^{-2}	0	44
1 in 100,000	10^{-3}	3	12
1 in 1,000,000	10^{-4}	5	52
1 in 10,000,000	10^{-5}	14	57
1 in 100,000,000	10^{-6}	33	51

It was also observed that a concentration of 1 in 100 i.e., 1 mg. in 0·1 c. c. of the venom solution required more than 2 minutes to coagulate 1 c. c. of the Fowl plasma. Work is proceeding.

XVIII. Chemical composition of urine of healthy Indians.

(GOKHALE.)

Examinations are done on 24-hour collections of urines of healthy subjects. The nitrogenous constituents, H ion concentration, total acidity, inorganic phosphate, calcium, chlorides, etc., are being estimated. The results of the few examinations done so far show that the urines are definitely low in nitrogenous contents as compared with those given in literature for European and American subjects.

XIX. Evolution of Hydrocyanic acid gas from Cymag.

(GOKHALE.)

Observations were carried out to ascertain the concentration of HCN gas obtained from Cymag, a fumigant of the Imperial Chemical Industries (India) Ltd. The Cymag was pumped in

a semiglass chamber of a capacity of 200 cubic feet by means of a Cynogas Duster (Foot Pump). Samples of air within the chamber were aspirated for estimation of HCN content from three different levels at different intervals of time ranging from 5 minutes to 4 hours from the time the fumigant was pumped in. The results are given in the following table:—

Amount pumped in.	Theoretical concentration of HCN.	Mean Temperature O°C.		Time in minutes.								
				5	15	30	45	60	90	120	180	240
50·2 g. ...	1420 parts per million.	29·2 to 30·8.	HCN Concentration parts per million.	321	393	482	553	590	699	650	545	512
			Percentage of theoretical parts per million.	23	28	34	39	42	49	46	38	36
			Relative Humidity per cent.	65	66	64	65	66	65	65	62	60
56·4 g. ...	1560 parts per million.	28·5 to 29·0.	HCN Concentration parts per million.	243	276	279	287	370	475	452	403	348
			Percentage of theoretical parts per million.	16	18	18	18	24	31	30	26	22
			Relative Humidity per cent.	51	51	49	50	50	54	60	57	61
43·8 g. ...	1210 parts per million.	30·0 to 30·8.	HCN Concentration parts per million.	264	411	500	530	553	585	542	520	421
			Percentage of theoretical parts per million.	22	34	41	44	46	48	45	43	35
			Relative Humidity per cent.	72	70	69	70	68	69	65	64	62

The results show that the maximum concentration of HCN gas has not gone over 50 per cent. of the theoretical content, and that even this concentration has been reached after an hour and a half.

XX. The Cultural Requirements of the Plague Bacillus: the essential amino acids.

(Sadashiva Rao.)

Very little is known of the nutritional factors that are essential for the growth of the plague bacillus. A study of this aspect of nutrition was therefore made, using the same technique and on a similar line as that initiated recently by Fildes and his collaborators. The amino acids necessary for growth have been determined, under rigidly controlled conditions excluding the

presence of every unknown chemical compound. Throughly washed cells of three strains of plague (the I strains virulent and avirulent, and the H. 36 strain) were used in experiments on the ability of chemically defined or "synthetic" media to support growth. It was found that the bacillus grew well in a basal medium consisting of acid-hydrolysed gelatin, supplemented with Sorensen's M/30 phosphate buffer at pH 7·0, sodium citrate and magnesium sulphate The addition of possible sources of accessory growth factors, or vitamins, in the shape of diluted extracts of marmite, mare's urine, meat and blood to the basal medium had no stimulatory effect on growth. Tryptophane had no effect, while the addition of cystine, in general, gave a better growth. These facts pointed to the conclusion that no accessory growth factors were involved in the nutrition of the bacillus, and that the necessary amino acids (with the exception of cystine) were to be found among those occurring in gelatin. Experiments made to grow the organism in a mixture of pure amino acids met with entire success, and therefore confirmed these conclusions. All the three strains grew in a mixture consisting of the following 8 amino acids (supplemented with phosphate buffer, sodium citrate and magnesium sulphate) :—*dl*-alanine, *dl*-leucine, glycine, *dl*-proline and *dl*-cystine (M/1500 concentration), *dl*-phenylalanine, *d*-lysine and *d*-arginine (M/4000 concentration). Among these, glycine and all the racemic (*dl*-) amino acids were of synthetic origin while only two were from natural sources. Experiments were next made to determine which of the amino acids were indispensable by omitting each in turn from the full amino acid mixture. Proline and phenylalanine, both of which are amino acids having somewhat complex molecular structures, were found to be wholly indispensable. In the absence of cystine, only one strain grew. Glycine was nearly but not quite essential for two strains, while alanine appeared to be so for one strain. No significant difference in nutritional requirements, or of relative ease of growth could be detected between the virulent and the avirulent lines of the I strain. In conclusion, the plague bacillus, in spite of its purely parasitic nature, exhibits a relatively simple nutritional condition, particularly as it shows no dependence on accessory factors for growth. The work on nutrition is being extended, and further studies on metabolism associated with the growth and activity of the organism are being persued on biochemical lines.

XXI. Mosquito Larvicides.

(WATS.)

Although mineral oils have been used as mosquito larvicides with varying success, malariologists are not totally in favour of using oils as such, as they are slow to act, and the toxicity to mosquito larvæ of two otherwise similar oils may differ considerably.

This investigation was undertaken to discover a suitable toxic substance which added to the oil, produced rapid death in a large number of larvæ and pupæ within as short a time as possible.

At the outset it became necessary to determine what quantity of ordinary kerosene had to be used per square foot of water surface to obtain a cent. per cent., kill of larvæ and pupæ of Armigeres obturans, a culicine mosquito commonly obtainable in Bombay. This and all subsequent determinations were made in laboratory sinks and later confirmed in a concrete cistern. Chemicals were first tested in the form of emulsions as the saving of cost in the case of an emulsion is usually appreciable.

The reported success of the pyrethrum extract emulsions by Ginsburg in America and Covell in Delhi encouraged us to undertake the work. Among the substances tried as emulsions may be mentioned, pyrocide 20, a-dichlorobenzene, p-dichlorobenzene, napthalene, xylol, croton oil, etc. But the results obtained were disappointing. All emulsions tried, have a tendency to distribute throughout the volume of the water thus decreasing the surface concentration. The next step was to mix the same chemicals with kerosene as such without emulsifying. The mixture which proved most successful was that of cashew nut shell oil and kerosene oil. The results with substances other than cashew nut shell oil gave poor results as larvicides and a few that did work could not be used on account of their high cost.

Cashew nut shell oil stands out in a class by itself. It was found that whereas 3 ml. of kerosene oil alone had to be used per square foot of water surface, one ml. of a mixture of 5 parts of cashew nut shell oil and 95 parts of kerosene or High Speed Disel oil was adequate for the purpose. In addition to the laboratory experiments three field experiments carried out in agricultural wells have given most encouraging results.

A defect common to the use of all mineral oils and oil mixtures of larvicides is that the oil tends to be blown away from the surface as the treated area before many larvæ and pupæ are killed. This can be considerably remedied by using 1 per cent. stearic acid dissolved in the mineral oil. As the cost of the cashew nut shell oil is about Rs. 1-4-0 per gallon, a 5 per cent. solution in kerosene or H.S. Disel oil does not add appreciably to the cost specially when the expenses of the mixture made is three fold more than the mineral oils as such.

Larvicides Dry (in powder form).

Various substances were tried as such in powder form, the chief being Pyrocide (powder), Cuprous cyanide and calcium cyanide. Of these, Pyrocide was found to be ineffective and also prohibitive in cost. Cuprous cyanide mixed with some other inocuous powder to a concentration of 2 per cent. to 4 per cent. succeeded under the laboratory conditions but field experiments are not conclusive. Calcium cyanide is a powerful larvicide, but the obvious difficulty of handling it, and distributing it on large areas prevents its further trials.

XXII. Search for para-colon and allied coligroup bacilli in water and faeces.

(GORE.)

Clemesha, the author of the pioneer monograph on the Bacteriology of Water Supplies in the tropics attempted in 1907 to revive the use of glucose bile salt broth along with MacConkey's lactose bile salt broth, believing that the production of acid and gas in glucose broth inoculated with quantities of water samples which fail to show the production of acid and gas in the lactose broth inoculated with equal quantities, would serve as indicator of faecal pollution remoter than that shown by lactose fermentation. However very little work appears to have been carried out on these lines by water bacteriologists in the tropics.

Bacteria which ferment lactose necessarily ferment glucose but not *vice versa*. In 1902 the late Sir Alexander Houston, F.R.S., had suggested the use of broth as the primary culture for water samples and the production of indol in these primary cultures as indicators of true B. coli. Subsequent work on intestinal bacteria has shown that among intestinal bacteria capable of producing indol there are many which are capable of fermenting glucose but not lactose, though not as many as those which ferment lactose.

Just as Clemesha had tried to revive the use of the glucose broth MacConkey had suggested in 1901 with a view not to miss the detection of B. enteritidis, so did I in 1922 try to revive Houston's suggestion believing that like glucose fermentation indol would indicate the growth of either or both the above two classes of intestinal bacilli.

However the criterion of the utility of an indicator of the growth of the indicated bacilli in mixed cultures like those of the primary cultures of water consists in the isolation of the indicator bacilli from the primary cultures. In the case of the primary cultures made in lactose broth, lactose fermenting bacilli are almost invariably isolated the primary cultures showing the production of acid and gas. Such was not the case when the primary cultures were made in peptone water or glucose broth.

In the course of the present investigation it was discovered that the reason for the failure to detect indol producing bacteria in general, but those capable of fermenting glucose but not lactose in particular from the primary peptone water cultures which responded positively to the indol test was the fact that either indol producing bacilli which had grown were over-grown by other bacilli at the end of 24 hours period of incubation at 37°C. or the media used for the isolation of bacilli from the mixed cultures were unsuitable.

Repeated work has substantiated the above observation and I have succeeded in isolating such bacilli from sewage, water and pathological faeces. Since these bacilli are not capable of fermenting lactose and liable to occur in quantities of water smaller than those in which lactose fermenting bacilli are detected, I am led to agree will Col. Clemesha that search of these bacilli should be made in as routine a way as that for lactose fermenters.

I have now been able to show that this could be as easily effected as the lactose fermenters. Since 1925 I had been occasionally ascertaining the production of H_2S and Indol in the series of MacConkey broth cultures in the routine use for the determination of the coliaerogenes count, and had often observed a positive response to H_2S and Indol or only Indol in culture tubes showing the production of acid and gas or acid or neither of these two products. Recently, I have been able to isolate the non-lactose-fermenting bacilli referred to above.

In a few strains of those bacilli for which anti-sera were prepared we had found that a larger number of strains isolated from faeces agglutinated with an antiserum prepared from a faecal strain, than the number of strains isolated from water or sewage with the faeces strain serum. Further work on these lines is in progress.

In fine the work I am at present carrying out is more or less a revival of Clemesha's idea regarding the need of routine search of coli-group of bacilli which ferment glucose but not lactose. The search of this class of bacilli commonly called "paracolon" is neglected also in the study of the bacteriology of faeces. The results so far obtained lead me to believe that in the case of the bacteriological examination of water just as the lactose-fermentation test has been regarded as an indicator of the potential danger of epidemic intestinal diseases, so would the H_2S and Indol tests indicate the potential (or may be, the actual ?) danger of subacute and chronic gastro-intestinal disorders. Do these bacteria play any part either as indicators or instigators in the etiology of some, if not all, deficiency diseases, e.g., sprue, pernicious and some other anaemias in which gastro-intestinal disorders prevail.

LIST OF PAPERS PUBLISHED FROM THE HAFFKINE INSTITUTE DURING THE YEAR 1937.

1. *Bhagvat, K.*—"Enzyme method for estimation of adrenaline", Current Science, Vol. V, No. 12, p. 646.

2. *Dikshit, B. B.*—"A Preliminary note on the action of Plasmoquine in pregnancy." Record Mal. Survey of India, Vol. VII, No. 1, p. 77.

3. *Dikshit, B. B.*—"Pharmacology of plasmoquine with special reference to its action in pregnancy." Read before the Malaria Symposium held at Calcutta.

4. *Dikshit, B. B., and Mahal, H. S.*—"Choline esterase in toxæmia" Quarterly Journal of Experimental Physiology, Vol. 27, No. 1, p. 41.

5. *Gokhale, S. K.*—"Total Ascorbic acid content of Human Blood", sent to the Indian Science Congress for January 1938 Session. Proc. Indian Science Congress, 1938, p. 287.

6. *Mahal, H. S.*—"Anti-septics and Anthelminthics." Proceedings Indian Academy of Sciences, Vol. V, No. 5, Sec. B, p. 186.

7. *Mahal, H. S., and Dikshit, B. B.*—"Choline ester formation by Pancreas". Current Science, Vol. VI, No. 5, p. 219.

8. *Nandi, B. K.*—"A new colour test for Chromates and Dichromates." Current Science, Vol. VI, No. 4, p. 156.

9. *Sokhey, S. S., and Chitre, G. D.*—"L'Immunite des Rats sauvages de ('Inde vis-a-vis de la Peste'). Bull. mens. de L'Off. Internat. d'Hyg. pub. XXIX, 2093, 1937.

10. *Sokhey, S. S., Gokhale, S. K., Malandkar, M. A., and Billimoria, H. S.*—"Red Cells, Hæmoglobin, Colour Index, Saturation Index and Volume Index Standards", Part I, Normal Indian Men: A Study based on the examination of 121 men. Indian Journal of Medical Research, 25, 2505, 1937.

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12. *Sokhey, S. S., and Maurice, H.*—Sur les Pouvoirs protecteurs relatifs de Vaccines Antipesteux prepares les Uns au Moyen de Cultures tuees par la Chaleur les autres au Moyen de Cultures vivantes avirulentes. (On the relative protective Powers of Plague Vaccines made of Heat-killed Cultures and those made of Live Avirulent Cultures). Bull. mens. de l'Off. International d-Hyg. pub. XXIX, 505, 1937.

13. *Wats, R. C., and Bharucha, K. H.*—"Cashew Nut Shell Oil as a Mosquito Larvicide". Current Science, Vol. VI, No. 5, p. 216.

14. *Wats, R. C., and Dalal N. P.*—"Prophylaxis of Chicken-pox by Inoculation with Vesicular Fluid". Indian Medical Gazette, Vol. LXXII, No. 3, March 1937, p. 155.

15. *Wats, R. C., and Jaswant Singh.*—"An investigation into the Mosquitocidal Value of Indigenous Derris and other drugs". Records of Malaria Survey of India, 7th March 1937, p. 109.

16. *Wats, R. C., and Harbhagwan.*—"A Note on the Prevention of Hæmoglobinuria in P. Knowlesi Infections in S. Rhesus by Methylene Blue and its Curative Value when combined with Quinine Salts". Records of Malaria Survey of India, 7th June and September 1937, p. 179.

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8th October 1938.

PART III.

APPENDIX.

Statistics of Anti-Rabic Inoculation.

(Standard Tables approved for use in India.)

1936.

APPENDICES.

TABLE I.

A General Statement regarding Patients treated at the Institute and its Centres from 1922 to 1936.

Year.		Europeans.				Asiatics.				Europeans and Asiatics combined.			
		Total treated.	Total deaths.	Total death rate per cent.	Advice cases.	Total treated.	Total deaths.	Total death rate per cent.	Advice cases.	Total treated.	Total deaths.	Total death rate per cent.	Advice cases.
1922...	Institute	64	.....	.....	.....	365	4	1·04	175	429	4	0·89	175
	Centres	7	.....	.....	.....	236	.....	.....	.....	243	.....	.....	.....
	Combined	71	.....	.....	.....	601	4	0·66	175	672	4	0·61	175
1923...	Institute	115	.....	.....	59	730	10	1·37	359	845	10	1·18	418
	Centres	38	.....	.....	.....	1,521	12	0·78	260	1,559	12	0·76	260
	Combined	153	.....	.....	59	2,251	22	0·97	619	2,404	22	0·91	678
1924...	Institute	62	1	1·61	41	525	9	1·71	372	587	10	1·70	413
	Centres	85	.....	.....	.....	2,880	28	0·97	330	2,965	28	0·94	330
	Combined	147	1	0·67	41	3,405	37	1·08	702	3,552	38	1·06	743
1925...	Institute	42	.....	.....	27	583	6	1·02	219	625	6	0·96	246
	Centres	187	.....	.....	5	3,569	28	0·78	393	3,756	28	0·78	398
	Combined	229	.....	.....	32	4,152	34	0·81	612	4,381	34	0·79	644
1926...	Institute	60	.....	.....	51	588	5	0·87	440	648	5	0·76	491
	Centres	287	.....	.....	20	3,861	25	0·64	322	4,148	25	0·60	342
	Combined	347	.....	.....	71	4,449	30	0·67	762	4,796	30	0·60	833
1927...	Institute	51	.....	.....	37	678	6	0·88	468	729	6	0·82	505
	Centres	303	.....	.....	31	3,857	25	0·64	490	4,160	25	0·62	521
	Combined	354	.....	.....	68	4,535	31	0·68	958	4,889	31	0·61	1,026
1928...	Institute	39	.....	.....	40	679	6	0·88	418	718	6	0·83	458
	Centres	216	.....	.....	27	4,166	34	0·81	467	4,382	34	0·77	494
	Combined	255	.....	.....	67	4,845	40	0·82	885	5,100	40	0·78	952
1929...	Institute	35	.....	.....	43	650	5	0·77	522	685	5	0·73	565
	Centres	286	.....	.....	19	4,621	43	0·93	507	4,907	43	0·89	526
	Combined	321	.....	.....	62	5,271	48	0·91	1,029	5,592	48	0·89	1,091
1930...	Institute	26	.....	.....	34	710	.....	.....	401	736	.....	.....	435
	Centres	314	1	0·29	10	4,692	50	1·07	92	5,006	51	1·01	102
	Combined	340	1	0·29	44	5,402	59	1·093	493	5,742	51	0·89	537

1931...	Institute	...	41	.....	.....	42	1,620	3	0·29	394	1,061	3	0·28	436
	Centres	...	328	.....	.....	3	5,670	40	0·70	80	5,998	40	0·68	83
	Combined	...	369	.....	.....	45	6,690	43	0·64	474	7,059	43	0·60	519
1932...	Institute	...	40	.....	.....	52	1,207	3	0·24	457	1,247	3	0·24	509
	Centres	...	252	.....	.....	8	5,984	60	1·00	35	6,236	60	0·96	43
	Combined	...	292	.....	.....	60	7,191	63	0·87	492	7,483	63	0·84	552
1933...	Institute	...	59	.....	.....	62	1,535	2	0·13	722	1,594	2	0·12	784
	Centres	...	298	.....	.....	8	6,139	31	0·50	32	6,437	31	0·48	40
	Combined	...	357	.....	.....	70	7,674	33	0·43	754	8,031	33	0·41	824
1934...	Institute	...	45	.....	.....	49	1,785	4	0·22	665	1,830	4	0·21	714
	Centres	...	344	.....	.....	24	5,462	23	0·42	21	5,806	23	0·39	45
	Combined	...	389	.....	.....	73	7,247	27	0·37	686	7,636	27	0·35	759
1935...	Institute	...	70	.....	.....	64	2,202	4	0·18	593	2,272	4	0·17	657
	Centres	...	306	.....	.....	7	5,544	32	0·57	44	5,850	32	0·54	51
	Combined	...	376	.....	.....	71	7,746	36	0·46	637	8,122	36	0·44	708
1936...	Institute	...	81	.....	.....	55	2,250	2	0·08	567	2,331	2	0·08	622
	Centres	...	246	.....	.....	10	5,515	29	0·52	28	5,761	29	0·50	38
	Combined	...	327	.....	.....	65	7,765	31	0·39	595	8,092	31	0·38	660
Grand Total		...	4,327	2	0·04	828	79,224	529	0·66	9,873	83,551	531	0·63	10,701

TABLE II.

A General classification of Patients attending the Institute during 1936.

Numerator=Deaths. *Denominators*=Cases.

	Europeans.					Asiatics.				
	Class of case treated.					Class of case treated.				
	I	II	III	IV	Total.	I	II	III	IV	Total.
Number fully treated	56	7	16	2	81	71	167	1/1904	1/108	2/2,250
Percentage to total	69·2	8·6	19·8	2·4	...	3·2	7·4	84·6	4·8	...
Total deaths	...	...	...	...	...	...	...	1	1	...
Total death rate per cent.	...	...	...	...	...	...	...	0·05	0·91	0·08
Percentage of health returns					100					98·4
Number not fully treated (presumably at risk)					...					8
Advice cases (not at risk)					55					567
Cases excluded from the statistical tables for other reasons					...					...
Total ...					55					575
Grand Total ...					136					2,825

TABLE II (*a*).

A general classification of patients attending the Centres during 1936.

Numerators = Deaths. *Denominators* = Cases.

	Europeans.					Asiatics.				
	Class of case treated.					Class of case treated.				
	I	II	III	IV	Total.	I	II	III	IV	Total.
Number fully treated	158	34	42	12	246	219	850	11/3,313	18/1,133	29/5,515
Percentage to total	64·3	13·6	17·4	4·7	...	3·9	15·2	60·0	20·9	...
Total deaths	...	...	...	...	...	...	...	11	18	29
Total death rate per cent.	...	...	...	...	...	...	...	0·33	1·5	0·52
Percentage of health returns					91·4					81·9
Number not fully treated (presumably at risk)					2					1,021
Advice cases (not at risk)					10					28
Cases excluded from the statistical tables for other reasons					...					...
Total ...					12					1,049
Grand Total ...					258					6,564

TABLE III.

Classification of patients according to position of wounds, interposition of clothing and probability of rabies in the biting animal.

Numerators = Deaths. *Denominators* = Cases.

Institute.

A—Animal proved rabid.
B—Animal certified rabid.
C—Animal probably rabid according to history.
D—History of animal unknown.
E—Animal probably not rabid according to history.

	Europeans.							Asiatics.							Total	Death rate per cent.
	A	B	C	D	E	Total.	Death rate per cent.	A	B	C	D	E	Total.	Death rate per cent.		
Bitten on face	1	...	...	...	...	1	...	1/4	1	6	51	...	1/62	1·6	1/63	1·5
Bitten on trunk and extremities (bare skin).	...	...	12	8	...	20	...	1/37	8	382	1,167	...	1/1,594	0·06	1/1,614	0·06
Bitten on trunk and extremities (through clothing).	...	...	3	4	...	7	...	...	11	23	489	...	523	...	530	...
"Licks" on any part of the body.	48	4	...	1	...	53	...	42	24	...	5	...	71	...	124	...
Total ...	49	4	15	13	...	81	...	2/83	44	411	1,712	...	2/2,250	0·08	2/2,331	0·08
Percentage to total ...	60·4	4·9	18·5	16·2	...	...	...	3·2	1·9	18·2	76·7	...	..	...	...	...
Death rate ...	...	...	...	...	...	...	...	2·4	...	...	...	...	0·08	...	...	...

TABLE III (*a*).

Classification of patients according to position of wounds, interposition of clothing and probability of rabies in the biting animal.

Numerators = Deaths. *Denominators* = Cases.

Centres.

A—Animal proved rabid.
B—Animal certified rabid.
C—Animal probably rabid according to history.
D—History of animal unknown.
E—Animal probably not rabid according to history.

—	Europeans.							Asiatics.							Total.	Death rate per cent.
	A	B	C	D	E	Total.	Death rate per cent.	A	B	C	D	E	Total.	Death rate per cent.		
Bitten on face	...	...	2	1	...	3	...	...	1/8	7/151	1/117	...	9/276	3·2	9/279	3·2
Bitten on trunk and extremities (bare skin).	1	2	16	14	...	33	...	18	9	20/2,875	568	...	20/3,470	0·57	20/3,503	0·57
Bitten on trunk and extremities (through clothing).	...	4	22	20	...	46	...	...	...	891	631	...	1,522	...	1,568	...
"Licks" on any part of the body.	91	19	28	26	...	164	...	55	70	93	29	...	247	...	411	...
Total ...	92	25	68	61	...	246	...	73	1/87	27/4,010	1/1,345	...	29/5,515	0·52	29/5,761	0·50
Percentage to total ...	37·3	10·1	28·0	24·6	...	...	...	1·3	1·5	74·1	23·1	...	96·4	...	...	...
Death rate ...	...	...	...	...	...	...	...	...	1·1	0·67	0·07	...	...	...	...	...

TABLE IV.

Age and sex distribution of patients treated at the Institute.

Numerator = Deaths. Institute. *Denominators* = Cases.

Age in years.	Europeans.				Asiatics.				Europeans and Asiatics combined.	
	Males.	Females.	Total.	Death rate per cent.	Males.	Females.	Total.	Death rate per cent.	Total.	Death rate per cent.
0—4 ...	4	.....	4	.....	1/56	17	1/73	1·3	1/77	1·3
5—9 ...	5	3	8	.....	210	86	296	.....	304	.....
10—14 ...	4	1	5	.....	277	53	330	.....	335	.....
15—19 ...	3	.....	3	.....	175	29	204	.....	207	.....
20—29 ...	9	15	24	.....	497	73	570	.....	594	.....
30—39 ...	10	8	18	.....	1/346	69	1/415	0·24	1/433	0·23
40—49 ...	7	2	9	.....	140	63	203	.....	212	.....
50—59 ...	2	6	8	.....	61	38	99	.....	107	.....
60—69 ...	2	.....	2	.....	30	26	56	.....	58	.....
70 and over ...	.....	.....	.....	.....	4	...	4	.....	4	.....
Total ...	46	35	81	.....	2/1,796	454	2/2,250	0·08	2/2,331	0·08

TABLE IV-*a*.

Age and sex distribution of patients treated at the Centres.

Numerator = Deaths. *Denominators* = Cases.

Centres.

Age in years.	Europeans.				Asiatics.				Europeans and Asiatics combined.	
	Males.	Females.	Total.	Death rate per cent.	Males.	Females.	Total.	Death rate per cent.	Total.	Death rate per cent.
0—4 ...	4	4	8	.....	2/133	1/87	3/220	1·3	3/228	1·3
5—9 ...	28	11	39	.....	5/684	3/179	8/863	0·92	8/902	0·88
10—14 ...	12	3	15	.....	5/576	134	5/710	0·70	5/725	0·68
15—19 ...	7	7	14	.....	1/336	74	1/410	0·24	1/424	0·23
20—29 ...	64	12	76	.....	3/1243	276	3/1519	0·19	3/1595	0·18
30—39 ...	40	18	58	.....	1/771	1/178	2/949	0·21	2/1007	0·19
40—49 ...	16	10	26	.....	1/398	1/138	2/536	0·37	2/562	0·35
50—59 ...	3	3	6	.....	4/132	1/69	5/201	2·4	5/207	2·4
60—69 ...	3	1	4	.....	55	19	74	.....	78	.....
70 and over ...	.....	.....	.....	.....	23	10	33	.....	33	.....
Total ...	177	69	246	.....	22/4351	7/11,64	29/5515	0·52	29/5761	0·50

TABLE V.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Europeans.
Institute.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg	...	3	3	1	3	1	...	...	...	...	...	...	...	...	...	...	11	...
Trunk	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Arm	53	10	2	2	1	...	...	...	...	...	...	...	...	...	...	...	68	...
Multiple ...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
Face	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
Multiple face ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	53	14	6	3	4	1	...	...	...	...	...	...	...	...	...	...	81	...
Percentage death rate. ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Separate table for Europeans and Asiatics : Also for Institute and Centres :—

(V) Europeans—Institute.
(V-a) „ —Centres.
(V-b) Asiatics —Institute.
(V-c) „ —Centres.

TABLE V-a.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Europeans.

Centres.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg	...	11	11	9	6	2	1	...	1	...	...	...	...	...	...	...	41	...
Trunk	...	1	...	1	1	...	...	...	...	...	...	...	...	...	...	...	3	...
Arm	164	14	11	7	...	...	...	1	...	...	1	...	...	...	...	...	198	...
Multiple ...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	1	...
Face	...	...	1	1	...	...	...	...	1	..	...	...	...	...	...	...	3	...
Multiple face ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	164	26	23	18	7	2	2	1	2	...	1	...	...	...	...	...	246	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Separate table for Europeans and Asiatics : Also for Institute and Centres :—

(V) Europeans—Institute.
(V-a) ,, —Centres.
(V-b) Asiatics —Institute.
(V-c) ,, —Centres.

TABLE V-b.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.
Institute.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg ...	...	298	579	263	1/256	86	43	22	5	3	1	5	1	...	1	...	1/1563	0·06
Trunk ...		8	12	9	10	1	...	...	...	1	...	...	...	...	...	...	41	.....
Arm ...	71	114	151	64	54	33	18	7	6	2	3	2	...	...	1	1	527	.....
Multiple ...	...	...	1	7	6	8	13	6	3	3	4	1	1	...	...	4	57	.....
Face ...	...	17	15	4	1/4	3	2	1	1	...	...	...	...	...	...	...	1/47	2·1
Multiple face ...	...	...	1	...	...	3	1	2	2	2	...	1	...	...	...	3	15	.....
Total ...	71	437	759	347	2/330	134	77	38	17	11	8	9	2	...	2	8	2/2250	0·08
Percentage death rate. ...	...	...	...	...	0.60	...	...	...	...	...	...	...	...	...	...	...	...	.....

Separate table for Europeans and Asiatics : Also for Institute and Centres :—

(V) Europeans—Institute.
(V-a) „ —Centres.
(V-b) Asiatics—Institute.
(V-c) „ —Centres.

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TABLE V-c.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Centres.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg ...	...	1/442	1111	748	1/615	1/159	1/116	37	35	7	15	9	2	1	2	3	4/3302	0·12
Trunk ...	...	40	77	55	44	13	6	2	1	1/1	...	...	...	1	...	...	1/240	0·41
Arm ...	247	1/184	1/374	228	5/208	67	1/59	24	1/25	8	1/7	7	3	1	2	1/3	11/1447	0·70
Multiple ...	...	...	22	30	1/61	35	2/34	13	14	9	1/9	6	7	2	2	6	4/250	1·6
Face ...	...	37	1/39	1/33	1/33	1/13	1/8	1	2	...	...	...	...	...	...	1	5/167	2·9
Multiple face ...	...	...	8	12	18	1/10	16	9	1/11	1/5	6	1/3	3	2	1	5	4/109	3·6
Total ...	247	2/703	2/1621	1/1100	8/379	3/297	5/239	86	2/88	2/30	2/37	1/25	15	7	7	1/18	29/5515	0·52
Percentage death rate.	...	0·28	0·12	0·09	0·82	1·0	2·0	...	2·2	6·6	5·4	4·0	...	...	...	5·5	...	...

Separate table for Europeans and Asiatics ; Also for Institute and Centres.

(V) Europeans—Institute.
(Va) ,, —Centres.
(Vb) Asiatics—Institute.
(Vc) ,, —Centres.

TABLE VI.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Institute.

Deep Bare.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
+ +	...	7	1	2	2	...	...	...	...	...	...	...	...	...	...	...	12	...
+ −	...	...	...	...	1	1	...	...	...	...	...	...	...	...	...	...	2	...
− +	53	5	3	1	...	...	...	...	...	...	...	...	...	...	...	...	62	...
− −	...	2	2	1	...	...	...	...	...	...	...	...	...	...	...	...	5	...
Total ...	53	14	6	4	3	1	...	...	...	...	...	...	...	...	...	...	81	...
Percentage death rate	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VI-a.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Centres.

Deep Bare.	Number of wounds.																Total.	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
+ +	...	4	5	6	1	...	2	...	1	...	1	...	...	...	...	...	20	...
+ —	...	12	7	7	4	1	...	1	1	...	...	...	...	...	...	...	33	...
— +	161	7	6	2	...	1	...	...	...	...	...	...	...	...	...	...	180	...
— —	...	3	5	3	2	...	...	...	...	...	...	...	...	...	...	...	13	...
Total ...	161	26	23	18	7	2	2	1	2	...	1	...	...	...	...	...	246	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VI-b.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Institute.

Deep Bare.	Number of wounds.																Total	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
+ +	...	281	529	245	2/250	103	63	29	14	9	7	7	1	...	1	6	2/1545	0·12
+ —	...	70	132	82	64	30	12	8	2	2	1	2	1	...	1	2	409	...
— +	71	43	49	10	4	1	2	1	1	...	...	...	...	...	...	...	182	...
— —	...	43	49	10	12	...	...	...	...	...	...	...	...	...	...	...	114	...
Total ...	71	437	759	347	2/330	134	77	38	17	11	8	9	2	...	2	8	2/2250	0·08
Percentage death rate.	...	...	...	...	0·60	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VI-c.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Centres.

Deep Bare.	Number of wounds.																Total	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
+ +	...	3/299	2/312	1/738	8/668	3/200	5/163	61	2/60	2/23	1/25	1/20	1	4	5	1/14	28/3203	0·87
+ —	....	159	283	174	180	61	48	11	17	3	4	2	3	....	....	3	951	...
— +	247	115	216	78	53	16	10	8	4	3	1/4	2	...	1	1	...	1/758	0·13
— —	...	130	220	116	78	20	18	6	7	1	1	1	1	2	1	1	603	...
Total...	247	2/703	2/1631	1/1106	8/979	3/297	5/239	86	2/88	2/30	2/57	1/25	15	7	7	1/18	2/5515	0·52
Percentage death rate.	...	0·28	0·12	0·09	0·82	1·0	2·0	...	2·2	6·6	5·4	4·0	...	....	....	5·5	...	...

TABLE VII.

Classification according to number of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Institute.

Days late.	Number of wounds.																Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
0 ...	1	3	1	1	3	1	...	...	...	...	...	...	...	...	...	...	10	...
1 ...	4	1	2	2	...	...	...	...	...	...	...	...	...	...	...	...	9	...
2 ...	5	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	8	...
3 ...	11	2	...	1	...	...	...	...	...	...	...	...	...	...	...	...	14	...
4 ...	4	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	6	...
5 ...	1	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	3	...
6 ...	12	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	13	...
7 ...	3	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	...
8 to 14 ...	12	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	14	...
15 to 21 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
22 to 28 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
29 to 35 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
36 to 42 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
50 and over...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	53	14	6	4	3	1	...	...	...	...	...	...	...	...	...	...	81	...
Percentage death rate. ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VII-a.

Classification according to number of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Centres.

Days late.	Number of wounds.																Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
0 ...	50	3	1	3	...	..	...	...	1	...	...	...	...	...	...	...	58	...
1 ...	36	8	10	4	2	...	1	...	...	...	1	...	...	...	...	...	62	...
2 ...	13	6	7	3	2	1	1	...	1	...	...	...	...	...	...	...	34	...
3 ...	12	3	1	4	1	1	...	...	...	...	...	...	...	...	...	...	22	...
4 ...	23	3	1	1	...	...	...	...	...	...	...	...	...	...	...	...	28	...
5 ...	3	...	...	1	...	...	...	...	...	..	...	...	...	...	...	...	4	...
6 ...	11	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	13	...
7 ...	3	1	...	...	1	...	...	1	...	...	...	...	...	...	...	...	6	...
8 to 14 ...	7	1	2	1	...	...	...	...	...	...	...	...	...	...	...	...	11	...
15 to 21 ...	4	...	1	..	...	...	...	...	...	...	...	...	...	...	...	...	5	...
22 to 28 ...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
29 to 35 ...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	1	...
36 to 42 ...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
43 to 49 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
50 and over ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	164	26	23	18	7	2	2	1	2	...	1	...	...	...	...	...	246	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VII-b.

Classification according to number of wounds and lateness of arrival.

Numerators=Deaths. Asiatics. *Denominators*=Cases.

Institute.

Days late.	Number of wounds.																Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.		
0 ...	3	70	140	66	1/71	34	23	10	5	4	4	2	1	...	...	2	1/440	0·22
1 ...	4	180	341	159	1/124	60	32	21	10	7	2	6	...	...	1	3	1/953	0·10
2 ...	16	54	89	34	35	12	5	2	1	...	1	1	1	...	...	1	252	...
3 ...	...	20	41	20	20	7	3	1	1	...	1	...	...	...	1	...	118	...
4 ...	15	17	30	24	19	6	2	...	...	...	...	...	...	...	...	1	114	...
5 ...	7	18	19	6	13	3	2	2	...	...	...	...	...	...	...	...	70	...
6 ...	14	11	11	5	1	4	2	...	...	...	...	...	...	...	...	...	48	...
7 ...	4	8	10	7	3	1	...	...	...	...	...	...	...	...	...	...	33	...
8 to 14 ...	6	33	33	16	26	4	1	2	...	...	...	...	...	...	...	1	122	...
15 to 21 ...	2	16	17	6	11	1	2	...	...	...	...	...	...	...	...	...	55	...
22 to 28 ...	...	...	3	2	4	...	...	...	...	...	...	...	...	...	...	...	9	...
29 to 35 ...	...	4	5	2	1	...	...	...	...	...	...	...	...	...	...	...	12	...
36 to 42 ...	...	1	2	...	...	2	...	...	...	...	...	...	...	...	...	...	5	...
43 to 49 ...	...	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	3	...
50 and over ...	...	3	11	...	2	...	..	...	...	...	...	...	...	...	...	...	16	...
Total ...	71	437	759	347	2/330	134	77	38	17	11	8	9	2	...	2	8	2/2250	0·08
Percentage death rate.	...	...	...	...	0·60	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VII-c.

Classification according to number of wounds and lateness of arrival.

Numerators=Deaths. Asiatics. *Denominators*=Cases.

Centres.

Days late.	Number of wounds.																Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.		
0 ...	14	29	120	50	26	6	8	18	13	11	8	4	3	1	2	1	314	...
1 ...	64	128	179	225	1/203	2/101	103	22	31	1/8	13	10	2	3	2	4	4/1198	0·33
2 ...	25	187	1/210	1/198	4/255	74	1/59	10	1/21	1/9	1/8	10	5	1	1	4	10/1077	0·92
3 ...	28	1/76	360	187	1/99	1/15	3/11	13	12	...	3	...	...	1	...	1/2	7/807	0·86
4 ...	42	58	275	153	98	10	1/8	3	2	...	1	1/1	...	1	...	2	2/654	0·30
5 ...	12	1/32	105	82	46	18	9	4	1	...	1/2	...	1	...	...	...	2/312	0·64
6 ...	10	22	1/83	51	1/44	13	6	4	1	1	...	...	...	...	...	...	2/235	0·85
7 ...	3	32	50	31	21	8	2	...	2	...	...	...	1	...	...	1	151	...
8 to 14 ...	25	81	149	76	1/56	29	17	4	1	...	1	...	3	...	1	4	1/443	0·22
15 to 21 ...	3	29	74	31	15	8	...	...	...	...	...	...	...	...	...	...	160	...
22 to 28 ...	4	8	8	7	4	8	3	1	1	...	...	...	...	...	...	...	44	...
29 to 35 ...	7	5	3	2	4	4	8	7	1/3	1	1	...	...	...	...	...	1/45	2·2
36 to 42 ...	...	1	1	3	...	1	1	...	...	...	...	...	...	...	...	...	7	...
43 to 49 ...	1	3	7	1	...	1	2	...	...	...	...	...	...	...	1	...	16	...
50 and over ...	9	12	11	9	8	1	2	...	...	...	..	...	...	...	...	...	52	...
Total ...	247	2/703	2/1631	1/1106	8/979	3/297	5/239	86	2/88	2/30	2/37	1/25	15	7	7	1/18	29/5515	0·52
Percentage death rate. ...		0·28	0·12	0·09	0·82	1·0	2·0	...	2·2	6·6	5·4	4·0	...	...	...	5·5	...	...

TABLE VIII.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators=Deaths. Europeans. *Denominators*=Cases.

Institute.

Deep	Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+	+ ...	3	...	8	...	1	...	12	...
+	— ...	2	...	...	...	...	...	2	...
—	+ ...	1	...	60	1	...	...	62	...
—	— ...	5	...	...	...	...	...	5	...
Total ...		11	...	68	1	1	...	81	...
Percentage death rate.		...	...	...	...	...	...	...	...

TABLE VIII-a.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators=Deaths. Europeans. *Denominators*=Cases.

Centres.

Deep	Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+	+ ...	9	...	7	1	3	...	20	...
+	— ...	16	3	14	...	...	...	33	...
—	+ ...	5	...	175	...	...	...	180	...
—	— ...	11	...	2	...	...	...	13	...
Total ...		41	3	198	1	3	...	246	...
Percentage death rate.		...	...	...	...	...	...	...	...

TABLE VIII-b.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Institute.

Deep	Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+	+ ...	1/1074	9	365	45	1/37	15	2/1545	0·12
+	− ...	348	20	29	12	...	...	409	...
−	+ ...	44	...	128	...	10	...	182	...
−	− ...	97	12	5	...	...	...	114	...
Total ...		1/1563	41	527	57	1/47	15	2/2250	0·08
Percentage death rate.		0·06	...	...	...	2·1	...	...	...

TABLE VIII-c.

Classification according to position and depth of wounds, and interpositon of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Centres.

Deep	Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Precentage death rate.
+	+ ...	4/1846	1/120	10/845	4/170	5/126	4/96	28/3203	0·87
+	− ...	708	76	113	39	7	8	951	...
−	+ ...	296	12	1/406	19	20	5	1/758	0·13
−	− ...	452	32	83	22	14	...	603	...
Total ...		4/3302	1/240	11/1447	4/250	5/167	4/109	29/5515	0·52
Percentage death rate.		0·12	0·41	0·70	1·6	2·9	3·6	...	...

TABLE IX.

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Institute.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	5	...	5	...	...	...	10	...
1 ...	4	...	5	...	...	...	9	...
2 ...	1	...	6	1	...	...	8	...
3 ...	...	...	14	...	...	...	14	...
4 ...	...	...	6	...	...	...	6	...
5 ...	1	...	2	...	...	...	3	...
6 ...	...	...	13	...	...	...	13	...
7 ...	...	...	4	...	...	...	4	...
8 to 14 ...	...	...	13	...	1	...	14	...
15 to 21 ...	...	...	...	...	...	...	...	...
22 to 28 ...	...	...	...	...	...	...	...	...
29 to 35 ...	...	...	...	...	...	...	...	...
36 to 42 ...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	...
50 and over ...	...	...	...	...	...	...	...	...
Total ...	11	...	68	1	1	...	81	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE IX-(*a*).

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Centres.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	2	...	55	...	1	...	58	...
1 ...	11	2	47	...	2	...	62	...
2 ...	12	...	21	1	...	...	34	...
3 ...	5	...	17	...	...	...	22	...
4 ...	2	...	26	...	...	...	28	...
5 ...	1	...	3	...	...	...	4	...
6 ...	1	...	12	...	...	...	13	...
7 ...	2	...	4	...	...	...	6	...
8 to 14 ...	3	1	7	...	...	...	11	...
15 to 21 ...	1	...	4	...	...	...	5	...
22 to 28 ...	...	...	1	...	...	...	1	...
29 to 35 ...	1	...	1	...	...	...	1	...
36 to 42 ...	...	...	...	...	...	...	1	...
43 to 49 ...	...	...	...	...	...	...	...	...
50 and over ...	...	...	...	...	...	...	...	...
Total ...	41	3	193	1	3	...	246	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE IX-(*b*).

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Institute.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	1/301	8	98	16	11	6	1/440	0·22
1 ...	684	18	196	24	1/22	9	1/953	0·10
2 ...	163	5	73	5	6	...	252	...
3 ...	91	1	23	...	3	...	118	...
4 ...	71	1	37	2	3	...	114	...
5 ...	44	1	22	2	1	...	70	...
6 ...	26	...	21	1	...	...	48	...
7 ...	23	...	10	...	...	...	33	...
8 to 14 ...	85	5	26	5	1	...	122	...
15 to 21 ...	40	1	12	2	...	...	55	...
22 to 28 ...	6	...	3	...	...	...	9	...
29 to 35 ...	11	...	1	...	...	...	12	...
36 to 42 ...	5	...	...	...	...	...	5	...
43 to 49 ...	2	...	1	...	...		3	...
50 and over ...	11	1	4	...	...	...	16	...
Total ...	1/1563	41	527	57	1/47	15	2/2230	0·08
Percentage death rate.	0·06	...	...	...	2·1	...	...	...

TABLE IX-(*c*).

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Centres.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	183	15	86	14	12	4	314	...
1 ...	728	47	1/303	54	1/40	2/26	4/1198	0·33
2 ...	1/657	1/48	2/259	2/50	3/40	1/23	10/1077	0·92
3 ...	2/500	41	3/195	2/44	13	14	7/807	0·86
4 ...	359	27	1/196	40	16	1/16	2/654	0·30
5 ...	1/191	11	1/83	16	9	2	2/312	0·64
6 ...	143	13	1/52	4	1/13	10	2/235	0·85
7 ...	76	8	50	9	1	7	151	...
8 to 14 ...	280	17	1/123	7	12	4	1/443	0·22
15 to 21 ...	104	7	41	3	5	...	160	...
22 to 28 ...	24	...	15	3	2	...	44	...
29 to 35 ...	18	3	1/18	1	3	2	1/45	2·2
36 to 42 ...	4	1	2	...	...	...	7	...
43 to 49 ...	11	1	3	1	...	...	16	...
50 and over ...	24	1	21	4	1	1	52	...
Total ...	4/3302	1/240	11/1447	4/250	5/167	4/109	29/5515	0·52
Percentage death rate.	0·12	04·1	0·70	1·6	2·9	3·6	...	...

TABLE X.

Deaths from Hydrophobia amongst persons treated in

Europeans.

Institute.

Serial Number.	Case Number.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and position of wounds.					Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
Nil																					

TABLE X-a.

Deaths from Hydrophobia amongst persons treated in

Europeans.

Centres.

Serial Number.	Case Number.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and position of wounds.					Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
Nil																					

TABLE X-b.

Deaths from Hydrophobia amongst persons treated in

Asiatics.

Institute.

Serial Number.	Case No.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and position of wounds.					Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
1	2602	20	M.	4	Dog ...	A	...	...	...	4	4	+	−	+	+	1	1	2 2	F3 IV	5 % 7cc.	14 days
2	2854	30	M.	30	Do. ...	A	4	...	...	...	4	−	−	+	+	0	2	2	III	„ 5cc.	„ „

TABLE X-c.

TABLE

Deaths from Hydrophobia

Asia

Cen

Serial No.	Case No.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and of wo		
					Kind.	Class.	Leg.	Trunk.	Arm.
									Died during
1	235	1	F.	12	Dog ...	C	6	...	...
2	67	4	M.	50	Do. ...	C	5	5	4
3	11	11	M.	30	Do. ...	D	...	...	...
4	419	3	M.	6	Do. ...	C	...	...	...
								Died within 15 days	
1	505	16	M.	14	Dog ...	C	...	...	4
2	125	16	M.	7	Jackal ...	C	5	...	...
3	327	17	F.	4	Do. ...	C	...	9	...
4	408	18	F.	40	Do. ...	C	...	...	7
5	135	19	M.	22	Dog ...	B	...	...	...
6	382	21	M.	25	Do. ...	C	...	...	17
7	823	23	M.	5	Do. ...	C	...	...	...
8	73	23	M.	10	Do. ...	C	...	...	10
9	300	27	F.	50	Jackal ...	C	...	...	4
								Died after 15 days	
1	813	27	F.	5	Dog ...	C	3	3	...
2	29	27	M.	22	Do. ...	C	...	...	4
3	594	31	M.	10	Jackal ...	C	3	2	2
4	11	33	M.	4	Dog ...	C	...	...	8
5	378	34	M.	9	Do. ...	C	...	...	4
6	21	35	M.	20	Do. ...	C	...	...	2
7	812	41	M.	12	Do. ...	C	3	...	3
8	135	43	M.	4	Do. ...	C	...	...	1
9	295	47	F.	5	Do. ...	C	...	...	...
10	37	49	M.	54	Do. ...	C	1	...	...
11	370	53	F.	6	Do. ...	C	...	...	4
12	371	55	M.	10	Do. ...	C	2	...	2
13	161	56	F.	30	Jackal ...	C	...	...	6

X-c.

amongst persons treated in

tics.

tres.

position unds.		Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
treat	*ment.*										Days.
...	6	+	—	+	+	23	2	26	III	5 %	5 cc. 3
6	20	+	—	+	+	2	4	10	IVf	,,	10 ,, 4
4	4	+	+	+	+	4	1	16	IVf	,,	10 ,, 11
4	4	+	+	+	+	1	3	17	IVf	,,	7 ,, 12
after	*treatment.*										
4	8	+	—	+	+	2	2	20	IVf	5 %	10 cc. 14
...	5	+	—	+	+	3	2	21	III	,,	3·5 ,, 14
...	9	+	—	+	+	2	1	20	III	,,	4 ,, 14
2	9	+	—	+	+	1	3	22	IVf	,,	10 ,, 14
6	6	+	+	+	+	2	2	33	IVf	,,	7 ,, 14
...	17	+	—	+	+	3	2	26	IVe	,,	7 ,, 14
4	4	+	+	+	+	2	1	26	IVf	,,	7 ,, 14
...	10	—	—	+	+	5	3	31	III	,,	5 ,, 14
1	5	+	—	+	+	1	1	29	IVf	,,	10 ,, 14
after	*treatment.*										
...	6	+	+	+	+	3	3	33	IVf	5 %	7 cc. 14
...	4	+	—	+	+	6	2	35	III	,,	5 ,, 14
4	11	+	+	+	+	4	3	38	IVf	,,	7 ,, 14
...	8	+	—	+	+	35	2	70	III	,,	5 ,, 14
...	4	+	—	+	+	1	2	37	IVe	,,	5 ,, 14
...	2	+	—	+	+	2	2	39	III	,,	5 ,, 14
...	6	+	—	+	+	3	2	46	IVe	,,	5 ,, 14
...	1	+	+	+	+	3	2	48	IVe	,,	5 ,, 14
3	3	+	—	+	—	3	3	52	IVf	,,	7 ,, 14
...	1	+	—	+	+	5	4	58	III	,,	5 ,, 14
...	4	+	—	+	+	2	3	58	IVe	,,	5 ,, 14
...	4	+	—	+	+	2	4	61	IVe	,,	5 ,, 14
...	6	+	—	+	+	4	3	63	IVe	,,	7 ,, 14

TABLE

Serial No.	Case No.	Number of days from commencement of treatment to onset of symptoms.	Sex	Age in years.	Biting animal.		Number and of wo		
					Kind.	Class.	Leg.	Trunk.	Arm.
							Died after 15 days		
14	132	60	M.	30	Dog ...	C	...	...	....
15	704	62	M.	40	Do. ...	C	...	...	4
16	591	75	M.	17	D ...	C	4	...	...
17	740	83	M.	55	Do. ...	C	....	...	4
18	193	98	M.	50	Do. ...	D	...	...	...
19	111	122	M.	50	Do. ...	C	1	...	9
20	50	208	M.	9	Jac al ...	C	6	...	...

X-c—*contd.*

position unds.		Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
after	*treatment*—	co	ntinu	ed.							
5	5	+	+	+	+	1	3	64	IV-F	5 %	10cc. Days. 14
...	4	+	—	+	+	10	2	74	III	„	5 „ 14
...	4	+	—	+	+	2	2	79	III	„	5 „ 14
...	4	+	—	+	+	3	3	89	III	„	5 „ 14
2	2	+	—	+	+	6	2	106	IV-F	„	10 „ 14
...	10	+	—	+	+	2	3	127	IV-E	„	7 „ 14
...	6	+	—	+	+	3	3	214	III	„	3·5 „ 14

TABLE

Classification of the animal which bit,

Numerators = Deaths.

Animal.	Institute.								
	Number treated.			Percentage to total.			Death rate per cent.		
	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.
Dog ...	77	2/2,181	2/2,258	95·0	96·9	96·7	...	0·09	0·08
Jackal ...	2	17	19	2·6	0·7	0·8	...	...	...
Cat ...	1	28	29	1·2	1·2	1·2	...	...	...
Monkey ...	1	20	21	1·2	0·8	0·9	...	...	...
Fox ...	...	.....	.....	...	...	...	...	...	...
Man ...	...	3	3	...	0·1	0·1	...	...	...
Donkey ...	...	.....	.....	...	...	...	...	...	...
Horse ...	...	1	1	...	...	...	...	...	...
Buffaloes ...	...	.....	.....	...	...	...	...	...	...
Hyena ...	...	.....	.....	...	...	...	...	...	...
Wolf ...	...	.....	.....	...	...	...	...	...	...
Cow ...	...	.....	.....	...	...	...	...	...	...
Goat ...	...	.....	.....	...	...	...	...	...	...
Leopard ...	...	.....	.....	...	...	...	...	...	...
Pig ...	...	.....	.....	...	...	...	...	...	...
Panther ...	...	.....	.....	...	...	...	...	...	...
Camel ...	...	.....	.....	...	...	...	...	...	...
Bullock ...	...	.....	.....	...	...	...	...	...	...
Total ...	81	2/2,250	2/2,331	...	...	...	...	...	...

XI.

scratched or licked the patient.

Denominators = Cases.

Centres.								
Number treated.			Percentage to total.			Death rate per cent.		
Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.
242	22/4,858	22/5,100	98·4	88·0	88·0	...	0·45	0·43
...	7/553	7/553	...	10·5	9·5	...	1·2	1·2
...	13	13	...	0·2	0·2	...	...	...
1	11	12	0·4	0·1	0·2	...	...	...
...	1	1	...	.....	.....	...	...	...
2	34	36	0·8	0·6	0·6	...	...	...
...	14	14	...	0·2	0·2	...	...	...
1	17	18	0·4	0·3	0·3	...	...	...
...	1	1	...	.....	.....	...	...	...
...	.....	.....	...	.....	.....	...	...	...
...	.....	.....	...	.....	.....	...	...	...
...	5	5	...	0·8	0·7	...	...	...
...	1	1	...	.....	.....	...	...	...
...	.....	.....	...	.....	.....	...	...	...
...	2	2	...	.....	.....	...	...	...
...	2	2	...	.....	.....	...	...	...
...	1	1	...	.....	.....	...	...	...
...	2	2	...	.....	.....	...	...	...
246	29/5,515	29/5,761	...	.....	.....	...	...	...

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TABLE XII.

Number of patients treated by months.

	Institute.			Centres.			Institute and Centres combined.		
	Europeans.	Asiatics.	Total.	Europeans.	Asiatics.	Total.	Europeans.	Asiatics.	Total.
January ...	15	194	209	34	455	489	49	649	698
February ...	6	187	193	8	433	441	14	620	634
March ...	1	233	234	16	497	513	17	730	747
April ...	22	241	263	6	426	432	28	667	695
May ...	9	213	222	11	499	510	20	712	732
June ...	2	188	190	18	409	427	20	597	617
July ...	10	175	185	32	421	453	42	596	638
August ...	5	174	179	26	438	464	31	612	643
September ...	2	171	173	13	421	434	15	592	607
October ...	6	160	166	39	530	569	45	690	735
November ...	2	132	134	21	457	478	23	589	612
December ...	1	182	183	22	529	551	23	711	734
Total ...	81	2,250	2,331	246	5,515	5,761	327	7,765	8,092

TABLE XIII.

Statement showing brains submitted and examined for rabies.

1936.

Serial No.	Animal.	Number of brains examined.	Positive.	Negative.	Unfit for examination.
1	Dogs	210	165	41	4
2	Jackals	1	...	1	...
3	Monkey	1	...	1	...
4	Cows	3	3	...	...
5	Buffaloes	2	1	1	...
6	Human beings	1	1	...	...
	Total ...	218	170	44	4

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